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A Procedural Manual for the Large-Scale Rearing of the Biting Midge, *Culicoides variipennis* (Diptera: Ceratopogonidae)

A Procedural Manual for the Large-Scale Rearing of the Biting Midge, *Culicoides variipennis* (Diptera: Ceratopogonidae)

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ABSTRACT

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This publication describes the specialized procedures and equipment used for the large-scale rearing of the biting midge, *Culicoides variipennis*. Brief sections cover classification, economic importance, association of field and laboratory bionomics, and problems with colonization. Detailed instructions are provided for producing and maintaining the eggs, larvae, pupae, and adults of *C. variipennis*. Information is given on how authorized educational, governmental, or industrial laboratories can obtain this insect vector for colonization or research use.

Keywords: Insecta, Diptera, Ceratopogonidae, *Culicoides variipennis*, biting midge, laboratory rearing, procedures, equipment.

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INTRODUCTION

There is a paucity of information on the epidemiology of many arthropod-borne viruses (arboviruses). Arboviruses and suspected arboviruses comprise a large group of animal and human viruses known or thought to be transmitted biologically among susceptible hosts by hematophagous arthropods in nature (Karabatsos 1985). The mission of the Arthropod-borne Animal Diseases Research Laboratory (ABADRL) of the Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), is to conduct basic and applied studies on the arthropod-transmitted diseases of domestic animals. The research involves studies on the virus-vector-host interrelationships of bluetongue (BLU) virus serotypes, the closely related epizootic hemorrhagic disease (EHD) virus serotypes, vesicular stomatitis virus, and other arboviruses with domestic and wild ruminants and arthropods. Information on the natural history of these interrelationships remains incomplete.

The laboratory colonization and large-scale rearing of the biting midge, *Culicoides variipennis* (Coquillett), are essential in studying this insect as a vector of several disease agents of veterinary importance and in evaluating methods of control. Recent developments have emphasized the significance of other insect colonies maintained at numerous educational, governmental, and industrial laboratories. For example, laboratory-reared insects are used to study insect life history; insect behavior; insect physiology; insecticides, repellents, and attractants; insecticide resistance; insect pathogens, parasites, and predators; insect transmission of plant and animal disease agents; host-plant resistance; biological control; genetic control; insect nutritional requirements; insect taxonomy and classification; and genetic engineering (Knipling 1966, 1984). The ever-increasing demand for large numbers of laboratory-reared insects has required the development of more efficient and economical methods of production. This manual describes the procedures and equipment used for the large-scale rearing of *C. variipennis* at ABADRL.

CLASSIFICATION

Culicoides variipennis was originally described as *Ceratopogon variipennis* by Coquillett in 1901. Kieffer (1906) synonymized this species as *Culicoides variipennis* (Coquillett). Wirth and Jones (1957) proposed the following five subspecies of *C. variipennis* in North America: *C. v. albertensis, C. v. australis, C. v. occidentalis, C. v. sonorensis*, and *C. v. variipennis*. Wirth and Morris (1985) recognized these five subspecies as allopatric or with overlapping distributions. Genetic studies have confirmed the existence of at least *C. v. occidentalis, C. v. sonorensis*, and *C. v. variipennis*, each of which may prove to be distinct species upon further analysis (Tabachnick 1990, 1992).

ECONOMIC IMPORTANCE

Culicoides variipennis is widely distributed in the United States, Canada, and Mexico (Wirth and Jones 1957). This minute blood-sucking midge, a serious pest of livestock (Jones 1961b, Jones and Akey 1977, Jones et al. 1981), is the principal vector of BLU viruses in sheep, cattle, and wild ruminants (Price and Hardy 1954, Foster et al. 1963, Jones 1965, Jochim and Jones 1966, Luedke et al. 1967, Foster et al. 1968, Jones and Luedke 1969, Jones et al. 1981) and of EHD viruses in deer and cattle (Foster et al. 1977, Jones et al. 1977). Bluetongue disease is a serious economic threat to the U.S. livestock industry. It causes mortality in sheep, reproductive impairment in sheep and cattle, and restrictions in the international movement of breeding livestock and germplasm (Callis 1985, Gibbs and Greiner 1988, Holbrook 1988).

ASSOCIATION OF FIELD AND LABORATORY BIONOMICS

The process of establishing and maintaining insect colonies successfully is predicated upon careful attention to a number of factors, including rearing facilities, microclimate (that is, environmental controls), rearing procedures, and nutrition. Needham (1937) identified the determinants of successful insect rearing as (1) food, (2) protection from enemies, (3) a suitable physical environment, and (4) fit conditions for reproduction. The National Academy of Sciences, National Research Council Subcommittee on Insect Pests (1969), stated that research toward mass production must feature the development of (1) inexpensive standardized foods or rearing media, (2) techniques for extracting insect stages from their media, (3) techniques for providing acceptable space for high-density insect populations, (4) full understanding of the chemical and physical stimuli regulating mating and oviposition, (5) rearing-room isolation and use of disinfectants in rearing rooms, and (6) maximum automation. Furthermore, the subcommittee stressed dependability, efficiency, and quality. Singh (1984) reported that successful rearing of insects depends on sound knowledge of insect biology, behavior, habitat, and nutrition. Also, an understanding of the mating habits, preoviposition and oviposition periods, fecundity, longevity, sex ratio, environmental requirements, and food and feeding preferences of the insect is necessary in developing rearing techniques (Singh 1984).

The successful rearing of *C. variipennis* has been the result of simulating many of the insect's natural conditions in the laboratory. Thus, a limited understanding of the field bionomics, such as larval habitats, larval feeding behavior, adult habitats, adult feeding behavior, and adult reproduction (Wirth and Bottimer 1956; Jones 1957, 1960, 1961a, 1964, 1966; Jones et al. 1969; Parker et al. 1977), was sufficient to allow the initial field populations and subsequent generations of *C. variipennis* to adapt to the imposed laboratory environment and artificial rearing conditions.

Colonization

Very few populations of field-collected *Culicoides* have been successfully cultured in the laboratory. The first reports of success were those of Downes (1950) and Megahed (1956), both of whom colonized and maintained *C. nubeculosus* (Meigen) for 9 yr. Jones colonized *C. variipennis* in 1957, and this is the only species of *Culicoides* that is still continuously maintained at large-scale production levels (Jones 1960, 1964, 1966; Jones et al. 1969; ABADRL, unpublished data; Hunt, unpublished data); the current production level at ABADRL is approximately 50,000 adults per wk. Additional species that have been successfully maintained as laboratory colonies for varying periods of time include *C. guttipennis* (Coquillett) for 1 yr (12–15 generations) (Hair and Turner 1966); *C. furens* (Poey) for 4 generations (Linley 1968); *C. arakawae* (Arakawa) for 4 yr (Morii and Kitaoka 1968); *C. guttipennis* for 5 mo (Gazeau and Messersmith 1970); *C. nubeculosus, C. riethi* Kieffer, and *C. variipennis* for over 5 yr (Boorman 1974); and *C. arakawae* for 6–7 generations and *C. schultzei* (Enderlein) for 5–6 generations (Sun 1974).

In 1956, Jones (1957) established several small colonies of *C. variipennis* from insects collected throughout Texas so that adequate studies could be conducted on its control as a vector of BLU viruses of sheep (Price and Hardy 1954). The colonies were maintained for up to 12 generations (approximately 12 months) at the USDA–ARS Livestock Insects Investigations Laboratory in Kerrville, TX. Later, Jones (1960) secured and maintained another colony of *C. variipennis* in 1957. The colony was started from wild larvae and pupae collected from a human-sewage site at a dwelling (Texas A & M Substation No. 14, Edwards County, TX). This colony was relocated to the USDA–ARS Animal Diseases Research Laboratory in Denver, CO, in 1962. The colony was transferred again when the USDA–ARS ABADRL (renamed in 1975) was moved to Laramie, WY, in 1985. This laboratory-reared insect is commonly referred to as the "AA" colony. The colony has been maintained without the addition of wild *C. variipennis* since December 1957. Genetic studies show that this colony is *C. v. sonorensis* (Tabachnick 1990).

After Jones established the second colony in 1957, a number of investigators have contributed further developments in large-scale production to standardize the insect rearing techniques. These significant developments include aeration of aquatic rearing medium, refrigerated egg storage, and volumetric measurement of pupae (Jones 1960); dispersal of bacterial scum in rearing medium, insect security precautions, and importance of uniform size of larvae (Jones 1964); nutritive ingredients, inert Dacron fibrous matting as substrate for immature aquatic stages, microbial-broth system, nutrient broth fluid concentrate for bacterial populations, and significance of photoperiod and of age of adults (Jones et al. 1969); modified apparatus for artificial blood feeding (Jones and Potter 1972); determination of optimal rearing temperatures and optimal larval density (Akey et al. 1978); a system for processing insect rearing data (Akey et al. 1984); use of lower adult holding temperature and importance of minimal handling of adults (Hunt et al. 1989); improved apparatus for artificial blood feeding and synthetic feeding membrane (Hunt and McKinnon 1990); and automated environmentally controlled insect rearing racks, deionized (DI) water as rearing medium, proper dietary formulations, injection-molded unplasticized polyvinylchloride (PVC) insect rearing pans, and volumetric measurement of larvae (ABADRL, unpublished data).

Longevity

Barnard and Jones (1980) observed that the generation time for *C. variipennis* was approximately 2 wk in Colorado. Mullens and Lii (1987) reported that 9–11 discrete generations of *C. variipennis* occurred per year in California. The generation interval estimate was 3.0–4.5 wk during summer and fall, whereas the winter generation duration was approximately 6–8 wk (Mullens and Lii 1987). Mullens and Rodriguez (1988) observed a generation interval of 6–7 wk during colder weather.

The natural longevity of adults is unknown because it is difficult to obtain accurate estimates of gonotrophic age. A few females have survived to complete four gonotrophic cycles and were estimated to be a minimum of 19 days old in New York State (Mullens 1983, Mullens and Rutz 1984).

The duration of the life cycle of *C. variipennis* in the laboratory at the time of colonization was approximately 30 days (Jones 1957, 1960). With the improvement of insect rearing techniques, Jones (1964) was able to reduce this life cycle to approximately 24 days. Currently, the life cycle is approximately 19 days or more and is as follows: egg, 2 days; larva, 10 or more days; pupa, 3 days; period prior to blood digestion, 1 day; and preoviposition, 3 days.

The longevity of female adults has been as long as 43 days under laboratory conditions (Jones 1964). Laboratory studies showed that mean and maximum longevities were significantly greater for female adults of *C. variipennis* held at 16.7 °C during the day and 8.9 °C during the night or at constant 15.6 °C compared with those held at 26.7/18.3 °C or 32.2 °C, respectively (O'Rourke and Washino 1981). Akey et al. (1978) demonstrated that larger female adults of *C. variipennis* survived 1½ times longer than smaller adults. The larger and more robust adults also produced more eggs.

Larval Habitats

Culicoides variipennis is very tolerant of differences in larval habitat; the larvae have been found in a wide variety of ecological habitats (for example, freshwater, salt water, or alkaline water) (Jones 1961a). Throughout North America, suitable habitats of this species consist of shallow, soft silt with standing or slow-flowing water; high concentrations of

minerals and organic matter (for example, human and livestock wastes); microorganisms; direct exposure to sunlight; and a lack of thick vegetation (Wirth and Jones 1957; Jones 1961a, 1964; Rowley 1965, 1967; Jones et al. 1969; Battle and Turner 1972; Kardatzke and Rowley 1971; Kline and Greiner 1985; Mullens and Rodriguez 1985; Kline and Greiner 1992). Larval habitats are frequently associated with localities where livestock feed and drink (Jones 1965). O'Rourke et al. (1983) listed 12 specific larval habitats for *C. variipennis*, representing a wide variety of manure-contaminated water sources. Manure-polluted water sources, such as dairy wastewater ponds, produce large populations of *C. variipennis* (Schmidtmann et al. 1983, Mullens and Lii 1987, Mullens and Rodriguez 1988, Mullens 1989). Mullen and Hribar (1988) reviewed the literature and summarized the larval habitats for *C. variipennis* as (1) general (that is, wide range of aquatic habitats, including margins of ponds and streams, pools, seepage areas, sloughs, ditches, and marshes), (2) animal manure or manure-contaminated substrates, and (3) inland alkaline, or saline ponds or pools.

The larval and pupal environments in the laboratory consist of thirty-three 24- by 24- by 2-inch injection-molded unplasticized PVC pans. Three pans (numbered 21–23) are located in a small insect rearing rack and are used for egg hatching; 18 pans (numbered 1–18) are located in a large rearing rack and are used for larval rearing; and 12 pans (numbered 24–35) are located in a small rearing rack and are used for pupal rearing. Two pans (numbered 19–20) located in the large rearing rack are unused. Approximately 2½ gal of DI water are used per pan as the rearing medium; this volume of rearing medium accommodates about 10,000 eggs, larvae, or pupae per insect rearing pan. Inert Dacron fibrous matting is used as the substrate for the immature aquatic stages. In addition, a photoperiod of 13:11 (L:D) hr, a water temperature of 26.5 °C ± 1 °C, aeration of the rearing medium, and dispersal of the bacterial scum in the rearing medium are necessary.

Larval Feeding Behavior

Kettle (1962) noted that larvae of *C. variipennis* have thickened pharynges to feed on algae, fungi, and bacteria. Rowley (1967) reported that algae and plant debris in the water appeared to be the food of *C. variipennis*. Parker et al. (1977) identified the microbial members that were used as a food source by *C. variipennis* in the field and in the laboratory as being mostly bacterial contaminants of polluted water. A mixture of microorganisms were observed from field-collected samples of substrate known to support immature stages of *C. variipennis* (Mullens and Rutz 1983, Vaughan and Turner 1987). Mullens and Rodriguez (1985) reported that larvae of *C. variipennis* sometimes ingest particulate organic debris. Hribar and Mullen (1991) examined the alimentary tracts of *C. variipennis* and found a variety of organisms, including diatoms, arthropods, bacteria, fungi, and oligochaetes.

Bacteria, other small microorganisms, and small pieces of detritus are the primary nourishment of *C. variipennis* larvae in the laboratory, even though algae and fungi were not readily available during early colonization work (Jones 1966). In addition, this primary nourishment is supplemented with two dietary formulations that provide proteins, carbohydrates, lipids, sterols, minerals, and vitamins for optimal larval growth (ABADRL, unpublished data).

Adult Habitats

Most species of *Culicoides* found outdoors appear to rest in damp, cool locations (Kettle 1962, 1977). Downes and Wirth (1981) stated that adults of Ceratopogonidae live mainly in moist areas around the larval habitat. Malloch (1915) determined that evergreens were the favorite resting places of *C. variipennis*. This species avoids going inside dwellings (Holbrook, personal communication 1986). *Culicoides variipennis* does not disperse far from its breeding sites (up to 3.2 km), although it may occasionally be carried passively by the wind for greater distances (Whitehead 1934, Jones and Akey 1977, Lillie et al. 1981, Zimmerman et al. 1982). Genetic studies have shown that some populations of *C. variipennis* are genetically isolated from nearby populations of this species only 200 m away (Tabachnick 1992).

In the laboratory, mixtures of male and female adults of *C. variipennis* are maintained in 1-gal cardboard ice cream containers inside an environmental growth incubator at 26.5 °C ± 1 °C and 40–50 percent relative humidity (RH), with a photoperiod of 13:11 (L:D) hr. Up to 1,500 adults are accommodated per container.

Adult Feeding Behavior

Most female species of *Culicoides*, which possess well-developed biting and sucking mouthparts (Jobling 1928; Snodgrass 1943, 1944), require a meal of blood for egg development (Downes 1958). All male species of *Culicoides* are phytophagous, and both sexes subsist on sugar and water, usually from the nectar of flowers (Downes 1958). Mullens (1985) demonstrated that adults of *C. variipennis* in California ingest simple sugars in nature, especially during the winter and spring. A significantly higher rate of sugar positivity was determined in parous female adults than in nulliparous female adults or male adults.

It is difficult to find engorged females of *C. variipennis* in the field to identify their natural host range. Furthermore, the quantity of blood ingested by these tiny insects is usually so small that even if it all could be extracted it is insufficient for determining the origin of the blood meal. Observations on the feeding habits of *C. variipennis* have been described. Jones (1959) reported that this biting midge readily feeds on cattle, mice, rabbits, and sheep in laboratory tests. *Culicoides variipennis* has been described as attacking cattle, deer, desert bighorn sheep, horses, humans, mules, rabbits, sheep, and

swine in nature (Malloch 1915, Whitehead 1934, Hair and Turner 1968, Foulk 1969, Jorgenson 1969, Tempelis and Nelson 1971, Jones et al. 1977, Schmidtmann et al. 1981, Mullens and Rutz 1984, Mullens and Dada 1992).

In the laboratory, male and non-blood-fed female adults of *C. variipennis* are provided with DI water for up to 4 days before their scheduled use for egg production or research. Blood-fed females are provided with 10 percent sucrose solution and DI water for 4 days prior to and during oviposition. If adults scheduled for research use are not used within 4 days, the insects are provided with 10 percent sucrose solution and DI water; approximately 24 hr before their use, DI water is substituted for the sucrose solution. An artificial apparatus for blood feeding is used to allow up to 2,500 female adults to feed on temperature-controlled (36.5 °C) defibrinated normal sheep blood for about 3 hr through a reinforced silicone membrane (Hunt and McKinnon 1990).

Adult Reproduction

In many species of *Culicoides*, mating in flight is common and is often accompanied by swarming of the insects; this swarming occurs in proximity to a conspicuous terrain marker, such as vegetation or the margin of a pond (Downes 1955, 1958). The swarms or aggregations are usually composed of males, with females entering the swarms to mate.

Jones (1964) demonstrated that both the males and females of the established laboratory colony of *C. variipennis* mate repeatedly, soon after emergence and periodically throughout their lifetime. Jones and Foster (1978) and Jones and Schmidtmann (1980) believed that field populations of *C. variipennis* may mate in a confined space, such as an adult holding container or a cone used for blood feeding, rather than requiring sustained flight achieved in swarming. Autogeny has not been documented with *C. variipennis*.

PROBLEMS WITH COLONIZATION

The large laboratory colony of *C. variipennis* at ABADRL has been maintained since 1957. This long duration of successful rearing under laboratory conditions is attributed to the detailed procedures and equipment described in this manual. However, similar rearing procedures have proved inadequate for maintaining many field populations of *C. variipennis* collected throughout the United States and brought into the laboratory (Jones and Tabachnick, personal communication 1987; Hunt, unpublished data). Two major problems in maintaining populations brought into the laboratory are insufficient rates of blood feeding and unsuccessful mating.

An artificial apparatus for blood feeding was modified and now has a simple design to heat and mix the blood meals and a greater surface area for insect feeding. Several types of natural and synthetic membranes were evaluated for the highest feeding rate, and a reinforced silicone membrane (Davis et al. 1983) was found to be especially suited for repeated use in colony maintenance and virus infection studies. Both of these improvements have increased the rate of blood feeding and the subsequent egg production of this insect (Hunt and McKinnon 1990).

A small number of field-collected *C. variipennis* possess the ability to mate in a confined space, for example, in an adult holding cage or a cone used for blood feeding (Jones 1966; Jones and Foster 1978; Jones and Schmidtmann 1980; ABADRL, unpublished data; Hunt, unpublished data); this characteristic is essential to the laboratory colonization of field populations. Attempts to observe swarming behavior in the laboratory proved unsuccessful (Hunt, unpublished data). In some insect species, mating can be accomplished by placing decapitated male adults in physical union with female adults. This procedure did not work for *C. variipennis* (Hunt, unpublished data). Male adults from the established colony are sexually aggressive in the laboratory and will mate with field-collected female adults. However, field-collected male adults are sexually inept in the laboratory. Attempts to bypass mating altogether to achieve fertilization by artificial fertilization or artificial insemination have been unsuccessful (Hunt, unpublished data).

Several environmental parameters, such as high temperatures and excessive handling of the insects during routine maintenance techniques, have contributed to high mortality rates in adult *C. variipennis* (Hunt et al. 1989). Changes in environmental conditions or genetic variability often occur during the process of establishing and maintaining insect colonies. Laboratory insects differ from their wild parent population in a variety of behavioral and physiological characteristics (Bartlett 1984, Joslyn 1984, Bartlett 1985). Jones (1964) observed periods of low insect productivity of *C. variipennis* after any radical change in rearing conditions or techniques.

PROCEDURES FOR LARGE-SCALE REARING

The immature stages (eggs, larvae, and pupae) and adults of *C. variipennis* (fig. 1) reared at ABADRL are used for (1) maintaining the continuity of the insect colonies, (2) conducting basic entomological research, (3) studying this insect as a vector of BLU viruses and EHD viruses, and (4) evaluating methods of control. The procedures and equipment used to produce and maintain these insect stages are given herein. For convenience, the production of adults will be described first.

Production of Adults

Preparation of adult holding cages

Note: The components of an adult holding cage are shown in figure 2.

1. Place a 3-inch-long piece of 2-inch-wide masking tape on the side of a cage near the middle and between any two small openings, write the appropriate cage number on the

Figure 1. Developmental stages of *Culicoides variipennis*. From left, egg, larva, pupa, female adult, male adult.

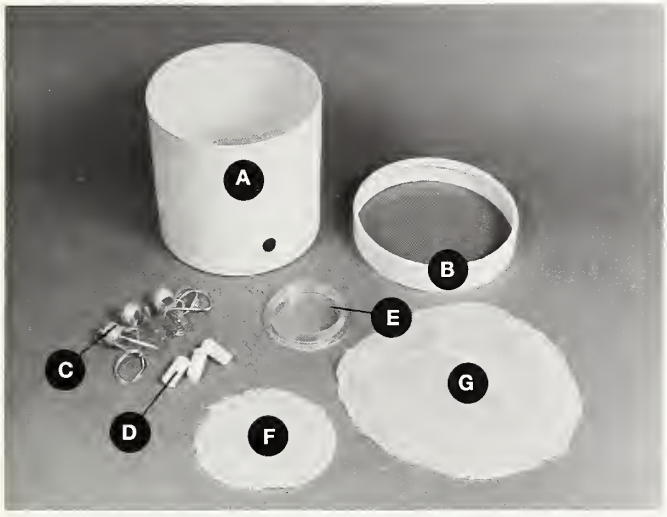

Figure 2. Components of an adult holding cage. *A*, 1-gal ice cream container. *B*, Container lid. *C*, Vials for DI water or 10 percent sucrose solution. *D*, Cotton wicks. *E*, Containers for oviposition and emergence. *F*, Small nylon organdy. *G*, Large nylon organdy.

tape, and record this number on form D111 *Colonies—Adult Production* (app. D).

2. Place a 9-inch-diameter piece of fine-mesh nylon organdy on top of the cage, and secure the material to the cage using the cage lid.

Note: The material should be taut when properly secured by the cage lid.

Caution: Make sure there are no openings around the cage lid for adults to escape through.

3. Fill three 2-dram lip vials with DI water, and insert a cotton wick into each vial.

4. Hold a vial upside down, move the wick back and forth until it is saturated, and allow approximately ¼ inch of the wick to protrude from the vial.

5. Insert the lip of each vial through the small openings of the cage, tilt the vials up at a 45° angle, and wrap the rubber bands around the perimeter of the cage (fig. 3).

Caution: Make sure that there are no openings around the vials for adults to escape through.

Handling of adult emergence

6. Insert an oviposition and emergence (O/E) container with its pupae (see steps 17–20 in *Production of Pupae*) through the bottom hole of a cage, and secure the container to the cage using three 7-inch-long pieces of 2-inch-wide tape placed in a parallel configuration to completely cover the bottom of the O/E container (fig. 4).

Note: Depending on the number of pupae collected, one to five cages may be prepared for adult emergence.

7. Place the cage(s) on a shelf located away from the air-circulating fans in the environmental growth incubator.

Note: Excessive air movement disrupts emergence activity.

8. During the following day, prepare additional cages for each cage that still contains viable pupae (see steps 1–5 in *Production of Adults*).

9. Monitor the temperature of the incubator daily, and record this temperature value on form D112 *Colonies—Physical Parameters* (app. D).

10. Remove all of the cages containing viable pupae from the incubator daily, and arrange the cages in numerical order.

Figure 3. Vial containing DI water or 10 percent sucrose solution being inserted through an opening of an adult holding cage

Figure 4. Oviposition and emergence container being secured to an adult holding cage

11. Handling one cage at a time, tilt and tap the side of the cage a few times so that the newly emerged adults (less than 24-hr old) fall to the bottom outer edge of the cage.

12. Immediately remove the old O/E container with its tape, insert an empty O/E container and a 5-inch-diameter piece of nylon organdy, and secure the new container using three new pieces of tape.

13. Add enough DI water in the old O/E container to remoisten the cotton substrate.

Caution: Do not add too much water, since the pupae will drown or will move out of the container and onto the bottom of the cage where they will desiccate.

14. Insert the old O/E container containing its viable pupae (that is, unemerged adults) into a new cage and secure the container using the same tape.

Note: The process of transferring an O/E container from one cage to the next is known as cropping.

15. Transfer the O/E container(s) from one cage to the next for 3 consecutive days.

16. During the fourth day, remove the cotton substrate containing pupal exuviae and a few viable pupae from the O/E container, place the substrate in the sink, run hot water on the substrate for a few minutes, and discard the material into an autoclavable waste bag.

17. Return the cropped cages to the incubator.

Note: The cropped cages may be placed on a shelf located near the air-circulating fans, since the air movement will not interfere with insect behavior.

Maintenance of adults

18. Remove all of the cropped cages from the incubator daily, and arrange them in numerical order.

19. Before cropping, remove a vial from its opening, and immediately cover the opening with a finger to prevent insects from escaping.

20. Hold the vial upside down and move the wick back and forth until it is moist.

21. Reinsert the vial into its opening, and similarly moisten the wicks of the remaining two vials.

Note: The vials must be refilled with DI water if they are empty. The process of moistening the wicks or refilling the empty vials is known as burping.

22. Burp the vials in the remaining cages, and return the cages to their proper locations in the incubator.

Cleaning of adult holding cages

23. Remove and discard the tapes.

24. Disassemble all components of the cages, discard the wicks and remaining DI water from the vials, and place the pieces of nylon organdy, O/E containers, and vials in a pan of warm water.

Caution: Do not use detergent during the cleaning process because the detergent leaves a residue that is toxic to the insects.

25. Wipe the O/E containers and the inside of the cages with a moistened sponge to remove dead adults, stains, and insect frass.

26. Clean the vials with a small radial-tip test tube brush and warm water.

27. Rinse the pieces of nylon organdy with warm water.

28. Allow the pieces of nylon organdy, O/E containers, and vials to air dry.

Production of Eggs

Blood feeding of adults

1. During blood-feeding days on Monday, Wednesday, and Friday, select adult holding cages from the incubator to be used for blood feeding, and record the cage numbers on form D111 *Colonies—Adult Production* (app. D).

Caution: If available, always save at least one cage containing 24- to 48-hr-old adults for emergency or unscheduled research use.

2. Place a 7½-inch-diameter carbon dioxide (CO_2) cover plate on a cage, open the valve to allow a slow release of the gas, and insert the gas line into the small opening of the plate for approximately 15 sec (fig. 5).

Note: A 15-sec exposure to the CO_2 anesthetizes the adults.

3. Remove the gas line and close the valve.

4. Tap on the side of the cage a few times to concentrate the adults, remove the cage lid and the 9-inch-diameter piece of fine-mesh nylon organdy, and dump the anesthetized adults through a small-size (4-inch diameter, 6⅛ oz) funnel and into a 25-ml graduated cylinder.

5. Anesthetize the adults in the remaining cages, and accumulate up to 5.0 ml of adults in the same cylinder.

Note: Usually three to four cages must be combined to accumulate 5.0 ml of adults. The 24- to 48-hr-old adults should be used first; the older adults should be discarded if they are not needed for blood feeding or research use.

6. Add up to 5.0 ml of adults from the cylinder into each cone used for blood feeding (fig. 6).

Note: A cone will accommodate only 5.0 ml of adults (approximately 2,500 females and 2,500 males) for a successful blood feeding. There are usually enough adults to fill two cones; if not, blood-fed female adults that have already laid eggs should be added to make up the difference.

Figure 5. Adults being anesthetized with carbon dioxide

Figure 6. Anesthetized adults being added to a cone used for blood feeding

7. Cover the opening of each cone with a 2- by 2-inch piece of hosiery, and secure it with two or more No. 8 rubber bands.

Caution: Make sure there are no openings around the top of each cone for adults to escape through.

Note: The anesthetized adults should recover within a few minutes.

Preparation of artificial apparatus for blood feeding

8. Secure a 2½- by 2½-inch piece of a reinforced silicone membrane to the bottom central well of each water-jacketed glass feeder with two or more No. 8 rubber bands (fig. 7).

9. Add DI water into the central well of each glassware unit; if leakage occurs, reattach or replace the membrane.

10. Place the two glass feeders in the openings of the plexiglass stand, and serially connect the flexible tubing to the inlet and outlet tubes of each glass feeder and to the constant-temperature water-circulating pump (fig. 8).

Figure 7. Reinforced silicone membrane attached to a water-jacketed glass feeder with small rubber bands

Figure 8. Water-jacketed glass feeder being placed on a plexiglas stand

11. Pour approximately 12 ml of sheep blood (held in the refrigerator) into each glass feeder.

Caution: Do not use sheep blood more than 10-days old because the red blood cells may have started to break down.

12. Place a stirring rod into each glass feeder, insert its plug into the variable-speed controller, and connect the electrical wires of the controller to the power supply (fig. 9).

13. Turn on the pump, add additional DI water into the pump reservoir (if necessary), displace air bubbles in the flexible tubing by squeezing the tubing, and wait approximately 10 min to allow the blood to reach the desired temperature of 36.5 °C.

Note: The 36.5 °C temperature is the natural skin temperature of sheep.

14. Turn on the power supply, and individually adjust the speed of the stirring rods to rotate at approximately 30 rpm.

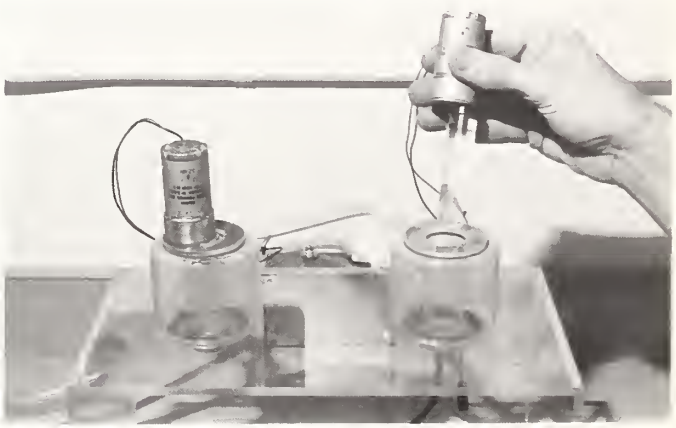

Figure 9. Stirring motor being placed in a water-jacketed glass feeder

15. Place the two cones on the adjustable-height platforms, and raise each platform until the top of each cone comes in contact with the membrane attached to the central well of the glass feeder (fig. 10).

Caution: Do not raise the platform any higher after contact has been established with the cone and the glass feeder because the glass feeder will be unstable for proper stirring action or the membrane on it may rupture.

16. Place the small metal partitions around each cone (fig.11).

Note: The partitions prevent air movement and high light intensity in the insect rearing laboratory from interfering with blood-feeding activity.

17. Allow the female adults to feed for approximately 3 hr.

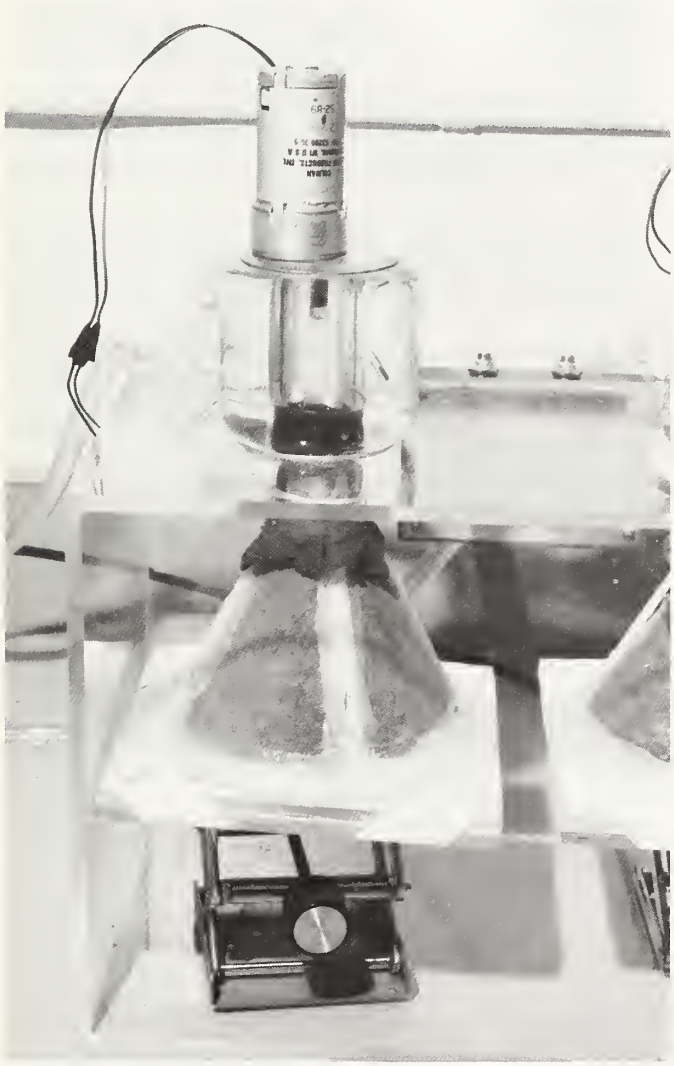

Figure 10. Cone used for blood feeding is positioned in contact with a water-jacketed glass feeder

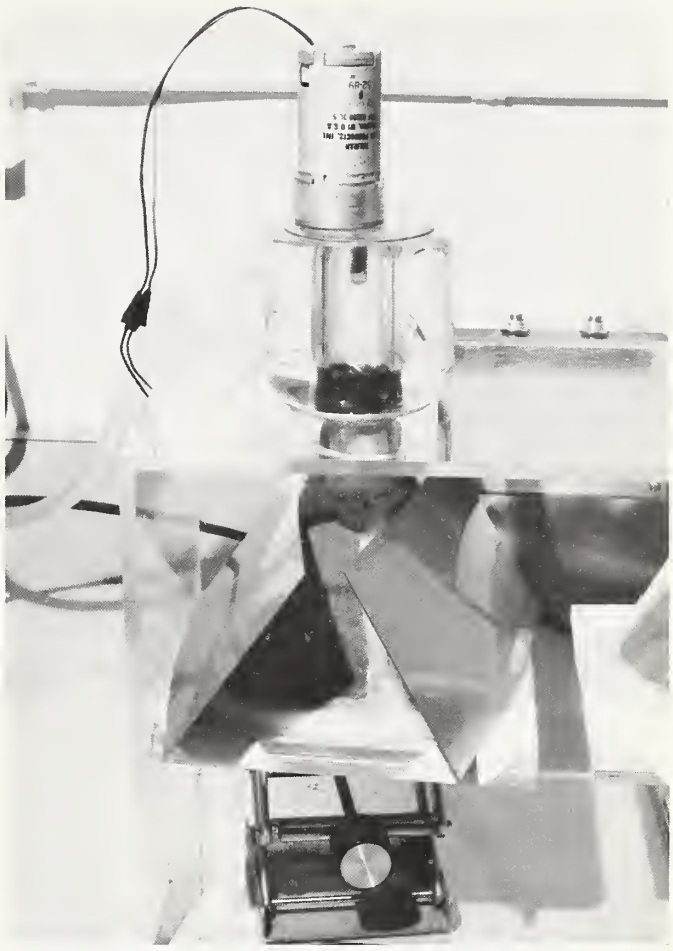

Figure 11. Metal partitions are placed around a cone used for blood feeding

18. Every ½ hr, lightly blow on the cones a few times to encourage blood-feeding activity.

Note: In nature, adults are attracted to the CO_2 produced by host animals during respiration; as the adults approach the animal, the CO_2 and several other factors stimulate blood-feeding activity.

19. After 3 hr, lower the platforms and remove the cones.

20. Place a medium-size (6-inch diameter, 16-oz) funnel upside down and over each cone, and use CO_2 for approximately 20 sec.

Note: It usually takes approximately 15 sec to anesthetize non-blood-fed adults and approximately 20 sec to anesthetize blood-fed adults.

21. Remove the funnel, small rubber bands, and hosiery; dump the anesthetized blood-fed adults into two new adult holding cages, and use a small paint brush to remove adults that are stuck inside each cone (see steps 1–5 in *Production of Adults*). However, use two vials of 10 percent sucrose solution and one vial of DI water per cage instead of three vials of DI water. Insert an empty O/E

container and a 5-inch-diameter piece of nylon organdy through the bottom hole of the cage.

Note: Allow the bottle of 10 percent sucrose solution (held in the refrigerator) to reach room temperature for approximately 30 min before preparing the two adult holding cages. Otherwise, the solution will drip onto the bottom of a cage when the cage is placed immediately in the warm incubator while the solution is still cold. Then, the adults will get stuck to the bottom of the cage and die. The sucrose solution provides a source of carbohydrates for the adults to prolong their longevity for oviposition.

Note: The O/E container and organdy allows no openings around the bottom hole for adults to escape through.

22. Attach a 3-inch-long piece of 2-inch-wide masking tape to each new cage, write the current Julian date and the appropriate symbol for the current day (for example, "A" for Monday, "B" for Wednesday, or "C" for Friday) on the tape, and record this information on form D114 *Colonies—Egg Production* (app. D).

23. Place the cages containing blood-fed adults on a shelf located away from the air-circulating fans in the incubator.

Note: The excessive air movement disrupts oviposition activity.

24. Clean the glass feeders and stirring rods with warm water, and then air dry them.

Collection of eggs

Note: The cages containing blood-fed adults are maintained for 4 consecutive days following the day of blood feeding.

25. Burp the vials containing 10 percent sucrose solution and DI water daily (see steps 19–22 in *Production of Adults*).

26. During the third day, prepare the oviposition substrates as follows: Fill two O/E containers with sterile cotton, soak the material with DI water, smooth out the surfaces with your fingers, drain the excess water, and place a 2¾-inch-diameter piece of filter paper on top of the moist material in each container.

Note: The thickness of the moist substrate should be approximately ⅜ inches.

Caution: If the substrate contains too much water, the adult insects will get stuck and die prior to oviposition. If the surface of the substrate is uneven, the egg distribution will not be uniform.

27. Remove the empty O/E containers and the 5-inch-diameter pieces of nylon organdy from the cage and replace these with the O/E containers accommodating the oviposition substrates.

28. During the fourth day, remove the O/E containers of eggs (fig. 12) from the cages, estimate the total number of eggs, and record these values on form D114 *Colonies—Egg Production* (app. D).

29. Place each oviposition substrate containing eggs into a disposable petri dish, add enough DI water to moisten the substrate, cover, secure the lid to the dish with a ½-inch-wide piece of masking tape, and write the current blood feeding day (that is, A, B, or C), the current Julian date, and the estimated number of eggs on the tape.

Caution: Fill the petri dish with DI water to a depth of only about ⅛ inch. If too much water is added, the eggs will float from the filter paper.

Note: The DI water provides a temporary rearing medium in case the eggs hatch during unfavorable temperature conditions that may occur, for example, if the refrigerator is inoperative.

30. Store the eggs in the refrigerator for up to 30 days, check the moisture level of the oviposition substrates daily and add DI water (if necessary), monitor the temperature of the refrigerator, and record this temperature value on form D112 *Colonies—Physical Parameters* (app. D).

Note: The viability and hatchability of the eggs under refrigerated conditions decrease significantly after 30 days (to less than 50 percent).

31. According to the status of insect production and the overall demand of the insect colonies, either destroy and discard the recently blood-fed adults (see step 32 below) or reuse the adults in combination with non-blood-fed adults for extra egg production (see step 33 on next page).

32. If the adults are not needed for further colony production, kill the adults by exposure to CO_2 and to running hot water, and clean the cages (see steps 23–28 in *Production of Adults*).

Figure 12. Oviposition substrates containing thousands of eggs

33. If the adults are to be reused for additional egg production, replace the old vials (two vials of sucrose solution and one vial of DI water) with three vials of DI water about 24 hr prior to the time the adults are scheduled for blood feeding.

Note: The absence of the sucrose solution will encourage the adults to readily blood feed the following day.

Production of Larvae

Preparation and maintenance of egg hatch pan

Note: The components of an egg hatch pan are shown in figure 13. There is a maximum capacity of three egg hatch pans (numbered 21–23) for the production of first- and second-instar larvae in the small insect rearing rack (fig. 14).

1. Cut three 4-inch-wide Dacron islands in half lengthwise, place the three pairs of 2-inch-wide islands into a clean insect rearing pan (24- by 24- by 2-inch plastic tray), and arrange these islands so that they are perpendicular to the front edge of the pan.

Caution: Make sure there is a 3-inch space between each pair of islands and between the islands and the sides of the pan to allow uninterrupted movement of water in the pan.

2. Split the front and back sections of each pair of islands approximately 2 inches deep, and insert a 4- by 2- by ½-inch stainless steel metal bar into each pair of split ends (fig. 15).

Note: The metal bars are used as weights to hold the islands in place while the water moves in the pan.

3. Place the pan on the top shelf of the small insect rearing rack, attach a paddle at both ends of the stirring rod so that a paddle sits at each edge of the pan (fig. 16), push the float unit and thermistor (if in use) down as far as possible (fig. 17), insert the plug of the automatic water-filling device into its proper socket to activate the automatic addition of DI water to the pan (fig. 17), and lay a thermometer in the water near the front edge of the pan.

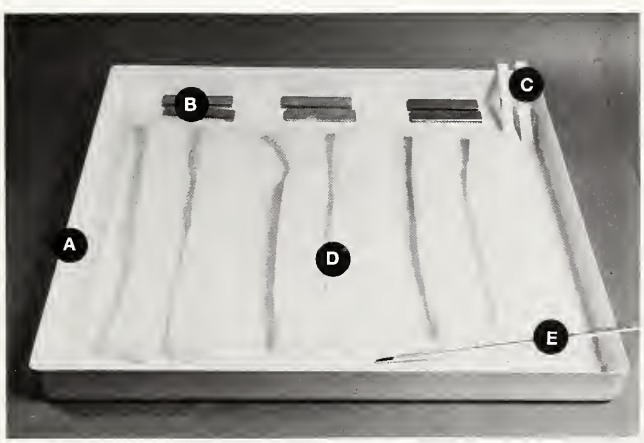

Figure 13. Components of an egg hatch pan with the proper arrangement of the Dacron islands. *A*, Insect rearing pan. *B*, Metal bars. *C*, Paddles. *D*, Narrow-width (2-inch) Dacron islands. *E*, Thermometer.

Figure 14. Insect rearing racks. Large rack is on left, small on right.

Figure 15. Stainless steel metal bar being inserted in a pair of Dacron islands of an egg hatch pan

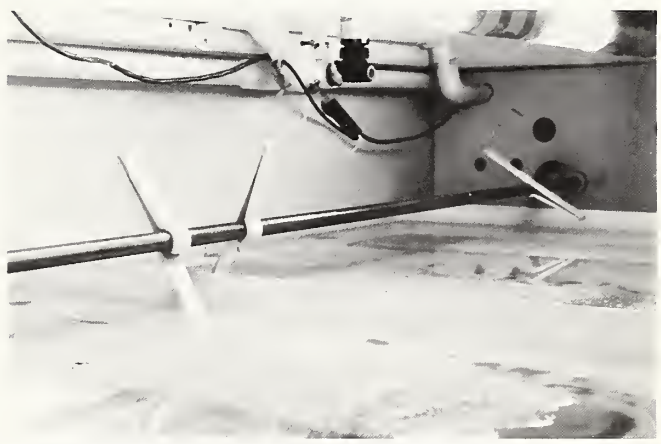

Figure 16. Paddles are attached on a stirring rod so that they are near the inner edges of an insect rearing pan

Note: The egg hatch pan is prepared on the top shelf of the small rearing rack to avoid the loss of eggs and small larvae that could result from a defective or malfunctioning water-filling device. The paddles help to minimize the formation of scum, a waste product of bacteria. The DI water level should be within ½ inch of the top edge of the pan.

Caution: Locate the islands at the water surface so that the larvae can easily maneuver on and off the islands. If the larvae cannot get back onto the islands because the islands are too high or submerged, the larvae will drown.

4. Add 6.0 ml of bacterial inoculum, 2.0 ml of nutrient broth fluid concentrate, and 2 level tsp of "Kalf" medium in the DI water.

Caution: Do not place these ingredients directly on the islands. Dispense the correct amount of dietary ingredients. Adding too much will produce a faster buildup of scum at the water surface and on the islands, and this scum will suffocate the larvae.

Note: The larvae feed on the microorganisms contained in the bacterial inoculum and on the particles of decaying organic matter in the pans.

5. Write the current and subsequent Julian dates and the preparer's initials on the egg hatch label below the pan (fig. 18), and record the pan number on form D116 *Colonies—Larval Production and Immature Use* (app. D).

6. Cut triangular-shaped sections from an oviposition paper; each section should contain approximately 10,000 viable eggs (fig. 19).

Note: A normal oviposition paper contains approximately 70,000 eggs laid from 2,500 female adults. The viability and hatchability of the eggs significantly decrease with age. The following information should be used to estimate viability:

Age of eggs (days)	Hatching rate (percent)
0–10	95
10–20	70
20–30	50

7. Carefully place one section of eggs on each island (fig. 20).

Caution: Do not submerge the sections in the water because the eggs will float away in the moving water or will not hatch properly.

Note: One egg hatch pan should be prepared every Wednesday, Thursday, and Friday.

8. To maintain the egg hatch pans, add 2.0 ml of nutrient broth fluid concentrate per pan each day for 6 days, and record this information on form D116 *Colonies—Larval Production and Immature Use* (app. D).

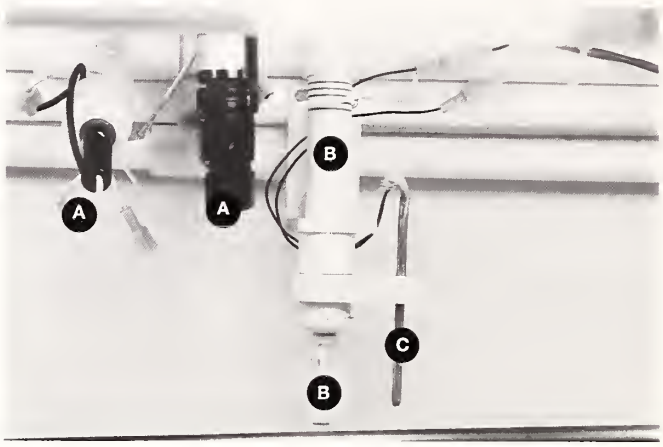

Figure 17. Device above each insect rearing shelf. *A*, Automatic water-filling device. *B*, Float unit (part of the automatic water-filling device). *C*, Thermistor.

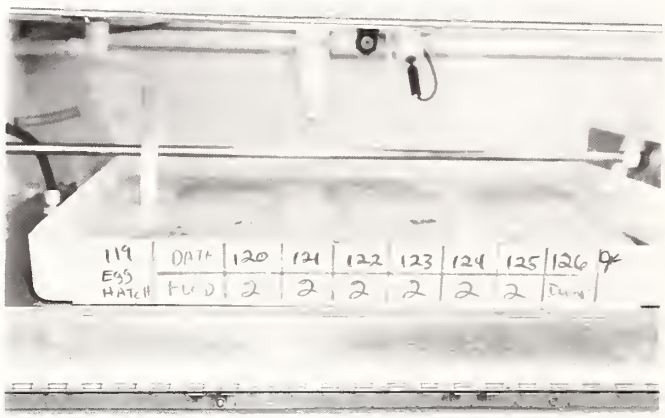

Figure 18. Egg hatch label listing the current and subsequent Julian dates and the preparer's initials

Figure 19. Triangular-shaped sections being cut from an oviposition paper

9. Monitor the temperature of the water in the hatch pans daily and record the values on form D112 *Colonies—Physical Parameters* (app. D).

10. Remove the scum that forms at the water level along and on the surface of the islands and along the edges of the pan with an aquarium algae scraper daily (fig. 21), and remove the scum on the paddles with a moistened sponge and a small radial-tip test tube brush.

Preparation and maintenance of larval rearing pans

Note: Figure 22 shows the components of a larval rearing pan. There is a maximum capacity of 18 larval rearing pans (numbered 1–18) for the production of third- and fourth-instar larvae in the large rearing rack (fig. 14).

11. Two days before the transfer of larvae from the egg hatch pan to the larval rearing pans, prepare six larval rearing pans in the large insect rearing rack by following the procedures used to prepare an egg hatch pan (see steps 1–5 in *Production of Larvae*); however, use two 4-inch-wide islands per pan instead of the six 2-inch-wide islands. Write the current and subsequent Julian dates and the preparer's initials on the larval rearing labels below the pans (fig. 23), and record the pan numbers on form D116 *Colonies—Larval Production and Immature Use* (app. D).

Figure 20. Triangular-shaped sections of an oviposition paper, each containing approximately 10,000 eggs and being placed on Dacron islands of an egg hatch pan

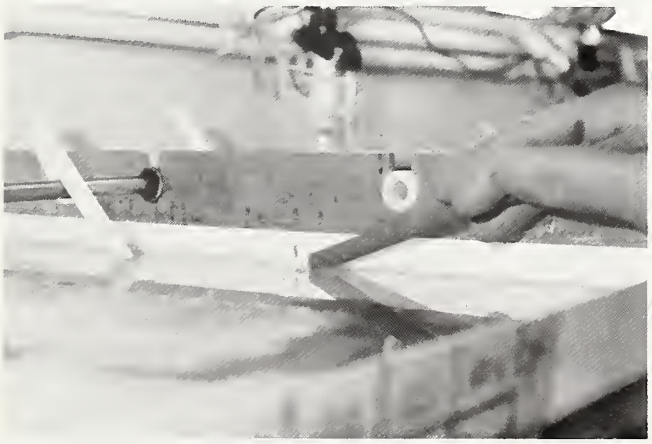

Figure 21. Aquarium algae scraper being used to remove scum from the inner edges of an insect rearing pan

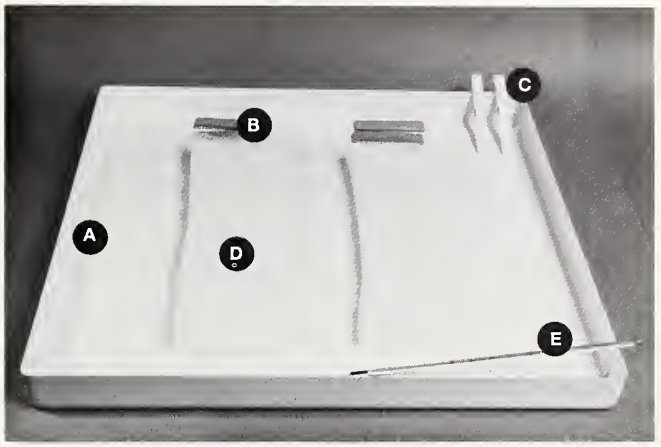

Figure 22. Components of a larval rearing pan with the proper arrangement of the Dacron islands. *A*, Insect rearing pan. *B*, Metal bars. *C*, Paddles. *D*, Medium-width (4-inch) Dacron islands. *E*, Thermometer.

Note: The 2-day duration allows the bacteria populations to reach desirable levels for optimal feeding by the larvae. The six larval rearing pans associated with one egg hatch pan should be prepared every Monday, Tuesday, and Wednesday. The thermometer should be used in those pans with a thermistor (that is, one thermistor per shelf in the large rearing rack).

12. To maintain the larval rearing pans, add 2.0 ml of nutrient broth fluid concentrate per pan on the second day, add ½ level tsp of "J" medium per pan on the third day (the day the larvae are transferred from the egg hatch pans to the larval rearing pans), add 3.0 ml of fluid concentrate per pan on the third through sixth days, and use form D116 *Colonies—Larval Production and Immature Use* (app. D) to record what and how much of each nutrient solution was added.

13. Monitor the temperatures of the water in the larval rearing pans daily, and record these values on form D112 *Colonies—Physical Parameters* (app. D).

14. Use a scraper daily to remove the scum that forms at the water level along and on the surface of the islands and along the edges of the pan, and remove the scum on the paddle with a moistened sponge and a test tube brush.

Transfer of larvae from egg hatch pan to larval rearing pans

Note: After 7 days of maintaining an egg hatch pan, the six islands should be transferred from an egg hatch pan to six larval rearing pans located in the large rearing rack. The steps that follow (steps 15–28) explain how to perform this transfer.

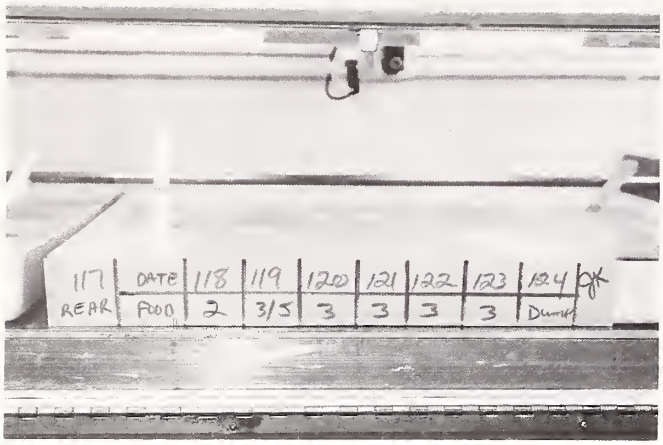

Figure 23. Larval rearing label listing the current and subsequent Julian dates and the preparer's initials

15. Remove the two paddles, six metal bars, and one thermometer from the egg hatch pan, and place these items in the sink so that they can be cleaned.

16. If the water-filling device is in use, disconnect its plug from the socket, and push the float unit and thermistor up as far as possible.

17. Roll up the six 2-inch-wide islands containing small (that is, first- and second-instar) larvae, use an enamel pan to hold the islands during the transport, and place and unroll one island between the pair of 4-inch-wide islands in each of the six larval rearing pans.

18. Place a 3-inch-wide flotation screen on each 2-inch-wide island, press down on the screen so that each island is submerged (fig. 24), and add extra DI water (if necessary) to completely submerge the islands.

Note: The small larvae will migrate from the submerged 2-inch-wide island to the two floating 4-inch-wide islands within 24 hr.

19. Place a large bucket in front of the small rearing rack and below the egg hatch pan.

20. Hold the draining tube (5/8-inch-diameter, 1/16-inch-thick flexible tubing) at the opposite end of the plastic U-shaped piece (two 90° 1/2-inch-diameter plumbing parts that are connected together), pinch the end of the tube closed, and fill the tube with tap water.

21. Place the plastic piece on the front edge of the pan, allow the other end to extend into the container, release the pinch, and allow the water to siphon from the pan into the container (fig. 25).

22. Place a No. 170 sieve in the sink, and slightly elevate a corner of the sieve with a large rubber stopper.

23. Remove the empty pan from the rearing rack, use the spray hose to wash any debris and remaining small larvae into a corner of the pan, and empty the contents of the pan into the sieve.

24. Pour the water in the container through the sieve.

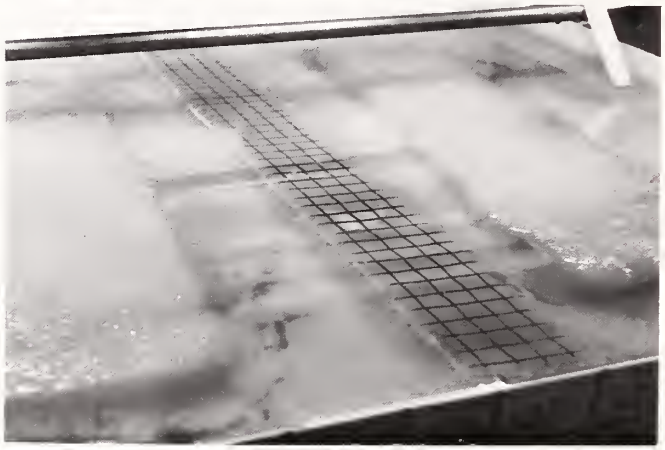

Figure 24. Flotation screen is placed on a Dacron island of a larval rearing pan

Figure 25. Water being drained from an egg hatch pan into a large container

25. Transfer the remaining small larvae to a 250-ml beaker, and distribute the contents of the beaker into the water of one recently prepared larval rearing pan.

Note: When transferring the six 2-inch-wide islands from an egg hatch pan to six larval rearing pans, not all of the small larvae will be on the islands—some larvae may be in the water; the sieve will collect any larvae left behind.

26. Clean the paddles, metal bars, thermometer, pan, draining tube, container, sieve, and beaker with a sponge and warm water; in addition, clean the empty shelf space, stirring rod, water-filling device, float unit, and thermistor (if in use) with a moistened sponge.

Caution: Do not use detergent during the cleaning process. Do not use hot water because it will deform the paddles.

27. Return the clean egg hatch pan and the accessories to their proper location in the small rearing rack, prepare the pan (see steps 1–7 in *Production of Larvae*), and record the pan number on form D116 *Colonies—Larval Production and Immature Use* (app. D).

Note: The 2-inch-wide islands should be transferred and an egg hatch pan should be cleaned and prepared every Wednesday, Thursday, and Friday to provide a continuous supply of insects.

28. During the following day on Thursday, Friday, and Saturday, remove the narrow-width screens from the six larval rearing pans, clean the screens with hot water, and air dry them; discard the 2-inch-wide islands into an autoclavable waste bag.

Collection of mature larvae from larval rearing pans

Note: After 7 days of maintaining the 6 larval rearing pans, the 12 islands in these pans should be transferred from the large rearing rack to the flotation tub to recover and concentrate the mature larvae. The components of the larval collecting system are shown in figure 26.

29. With the flotation tub elevated in the sink, place a stack of three sieves (consisting of a No. 140 sieve on the bottom, a No. 80 sieve in the middle, and a No. 70 sieve on top) under the drain tube of the tub.

30. Remove the paddles, metal bars, and thermometer (if in use) from each pan, and place the items in the sink so that they can be cleaned.

31. Disconnect the plug of the water-filling device from its socket, and push the float unit and thermistor (if in use) up as far as possible.

32. Roll up the 4-inch-wide islands containing large, mature (that is, third- and fourth-instar) larvae, and transport them in an enamel pan.

33. Lay six islands (with the larvae on top) in the bottom of the tub to form the first layer of islands (fig. 27), and use the six remaining islands to form the second layer above the first layer.

34. Drain one larval rearing pan at a time (see steps 19–21 in *Production of Larvae*), and pour the water into the tub until the water level in the tub reaches the small rubber stopper located in the drain tube (emptying four pans will usually fill the tub). Use your gloved fingers to sift through the islands, and then pour the water from the last two pans through the stacked sieves.

Note: Sifting through the islands allows the trapped larvae, which will drown after a few hours if not freed, to move freely to the water surface.

35. Place the three 5-inch-wide tub (or flotation) screens over the two layers of islands, push down to submerge the islands, and wedge the screens between the inside ridges of the tub (fig. 28).

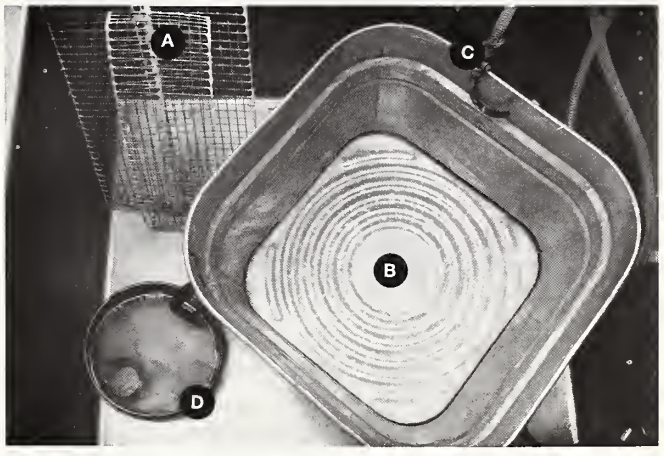

Figure 26. Components of a larval collecting system. *A*, Flotation screens. *B*, Flotation tub. *C*, Water hose. *D*, Sieves.

Figure 27. Dacron islands being placed on the bottom of the flotation tub

Caution: Do not push the islands too deep because the islands will compact and the larvae will be trapped so that they cannot maneuver to the water surface.

36. Position the utility light approximately 6 inches above the tub corner that has a rubber-stoppered outlet in it (fig. 29), turn the light on, and wait at least 30 min.

Caution: Use only a 40-W bulb in the utility light.

Note: The mature larvae will be attracted to the light and will congregate at the water surface along the sides of the tub. The 30-min period will allow the debris in the water to settle to the bottom and will allow the larvae easier access to the surface.

37. Place the hose in a position where it delivers temperature-controlled charcoal-filtered tap water just below the water level in the tub, and open the valve to produce a slow-moving stream (fig. 30).

38. Remove the small rubber stopper to allow the water to drain into and through the stacked sieves for approximately 1 hr.

Note: The floating larvae will drain into the appropriate sieves for collection.

39. While waiting, use a moistened sponge and warm water to clean the paddles, metal bars, thermometers (if in use), and pans; in addition, clean the empty shelf spaces, stirring rods, water-filling devices, float units, and thermistors (if in use) with a moistened sponge.

Caution: Do not use detergent during the cleaning process. Do not use hot water because it will deform the paddles.

40. Return the six clean larval rearing pans and the accessories to their proper locations in the large insect rearing rack, prepare the pans (see step 11 in *Production of Larvae*), and record the pan numbers on form D116 *Colonies—Larval Production and Immature Use* (app. D).

Figure 28. Flotation screens being placed over the 12 Dacron islands in the flotation tub

Figure 29. Utility light is placed near the flotation tub corner in which a rubber stopper is inserted

Figure 30. Temperature-controlled water hose is placed just below the water surface in the flotation tub

Note: The 12 medium-width islands should be transferred and the six larval rearing pans should be cleaned and prepared every Monday, Tuesday, and Wednesday to provide a continuous supply of insects.

41. After 1 hr, when most of the floating larvae have been collected or when the water appears to be clear, remove the water hose and reinsert the rubber stopper.

Caution: Do not exceed 1 hr because trapped larvae may drown before the second collection.

42. Remove the utility light and the three tub screens, and sort and invert the twelve 4-inch-wide islands.

Note: Sorting and inverting the islands frees the trapped larvae, especially those entangled in the bottom islands.

43. Transfer the larvae collected in the No. 70 sieve, the No. 80 sieve, and the No. 140 sieve to individually labeled 250-ml beakers.

44. Kill the remaining larvae in the islands with hot water, and discard the material into an autoclavable waste bag. Use a sponge and warm water to clean the tub, tub screens, bucket, and drain tube; air dry the cleaned components.

Caution: Do not use detergent during the cleaning process.

Volumetric measurement of mature larvae collected

Note: The components of the larval measuring system are shown in figure 31.

45. Place the 1,000-ml vacuum flask next to the ring stand.

46. Insert the larval measuring tube (a modified graduated cylinder) into the 5/8-inch-diameter, 1/8-inch-thick flexible piece of tubing at the top of the flask, use the adjustable stand clamp to secure the tube, and insert a small-size funnel into the measuring tube.

47. Connect the flexible tubing between the flask and the vacuum pump, and turn on the pump.

48. Slowly pour the contents of the 250-ml beaker containing the No. 70 sieve-size larvae into the funnel, and wait a few minutes for the pump to adequately remove the water from the measuring tube.

49. Estimate the volume (in milliliters) of larvae that are compacted in the measuring tube, record this quantity value on the form *Colonies—Larval Production* (app. D), and use the spray hose to force the compacted larvae out of the tube and back into their labeled 250-ml beaker.

50. Repeat steps 48 and 49 to determine and record the quantities of No. 80 and No. 140 sieve-size larvae and to transfer the compacted larvae back to their appropriately labeled 250-ml beaker; then turn off the pump.

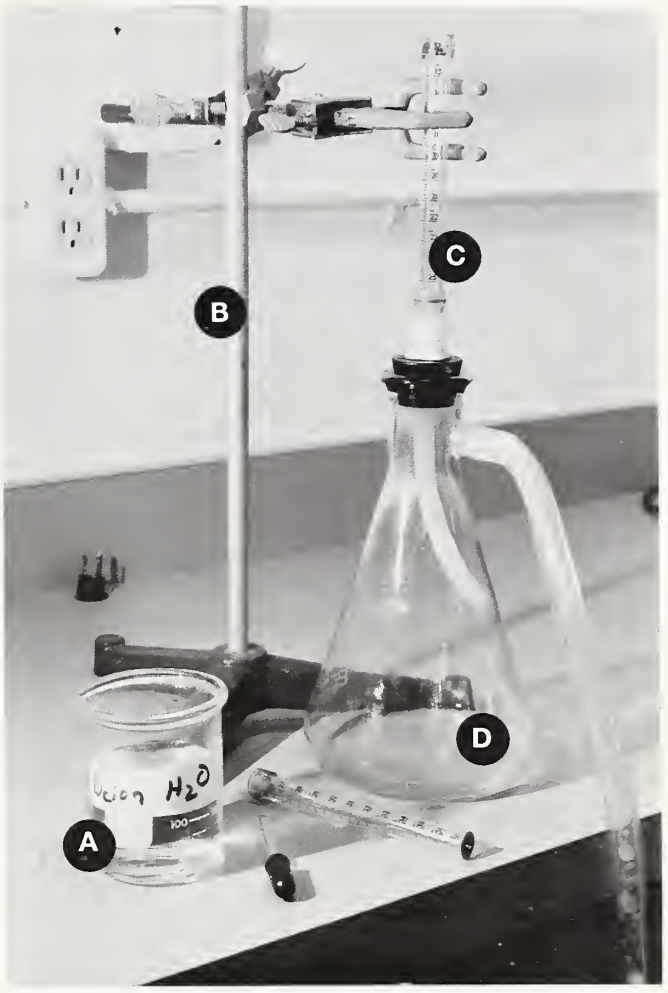

Figure 31. Components of the larval measuring system. *A*, Beaker of DI water. *B*, Ring stand. *C*, Larval measuring tube. *D*, Vacuum flask.

Note: There are approximately 2,000 No. 70 sieve-size larvae, 3,000 No. 80 sieve-size larvae, or 8,000 No. 140 sieve-size larvae per 1.0 ml. Large and robust adults will be produced when the optimal level of approximately 10,000 larvae per pan is achieved; these adults are advantageous for research and insect colony production because they will live longer and produce more eggs.

51. Since there are approximately 2,000 No. 70 sieve-size larvae per 1.0 ml, place up to 5.0 ml of these larvae on the 4-inch-wide islands of each of the two pupal rearing pans, and record these quantity values on the form *Colonies—Larval Production* (app. D) and on each pupal rearing label. If there is an excess of larvae collected in the No. 70 sieve, place these larvae into one or more of the recently prepared pupal rearing pans that did not reach the optimal level; otherwise, use hot water to discard the excess larvae.

Note: If there are less than 10,000 No. 70 sieve-size larvae collected per pan, the difference can be made up by adding some of the larvae collected in the No. 80 sieve to the two pans. Larvae collected in the No. 140 sieve can also be used to supplement the No. 70 sieve-size larvae if there are not enough No. 80 sieve-size larvae to bring up the total to 10,000. Excess larvae of any size should be discarded using hot water. The quantities of each size of larvae added to the rearing pans should be recorded on the label of the pupal rearing pan and on the form *Colonies—Larval Production* (app. D).

52. Disassemble the pieces of the larval measuring system, clean them with warm water, and air dry the equipment.

Note: The No. 70 sieve retains fourth-instar larvae; therefore, it takes approximately 1–7 days (generally with a peak at 4 or 5 days) for pupae to be produced from these larvae in the pupal rearing pans. The No. 80 sieve retains mostly third-instar larvae and some fourth-instar larvae; therefore, it takes approximately 3–14 days (generally with a peak at 8 or 9 days) for pupae to be produced from these larvae in the pupal rearing pans.

Production of Pupae

Preparation and maintenance of pupal rearing pans

Note: The components of a pupal rearing pan are the same as those of a larval rearing pan (fig. 22). There is a maximum capacity of 12 pupal rearing pans (numbered 24–35) for the production of pupae in the small insect rearing rack (fig. 14).

1. Two days before the transfer of third- and fourth-instar larvae from the six larval rearing pans to the two pupal rearing pans, prepare two pupal rearing pans in the small insect rearing rack by following the procedure used to prepare an egg hatch pan (see steps 1–5 in *Production of Larvae*); however, use two 4-inch-wide Dacron islands per pan instead of the six 2-inch-wide islands, and add 4 ml of nutrient broth fluid concentrate instead of 2 ml. Write the current and subsequent Julian dates and the preparer's initials on the pupal rearing labels below the pans (fig. 32), and record the pan numbers on form D116 *Colonies—Larval Production and Immature Use* (app. D).

Note: Two pupal rearing pans associated with the six larval rearing pans are cleaned and prepared every Saturday, Sunday, and Monday to provide a continuous supply of insects. The thermometer should be used in those pupal rearing pans having a thermistor (there should be one thermistor per shelf in the small rearing rack).

2. To maintain the pupal rearing pans, add 4.0 ml of nutrient broth fluid concentrate per pan on the second day, add 3 ml of fluid concentrate per pan on the third through fourteenth days, and record this information on form D116 *Colonies—Larval Production and Immature Use* (app. D).

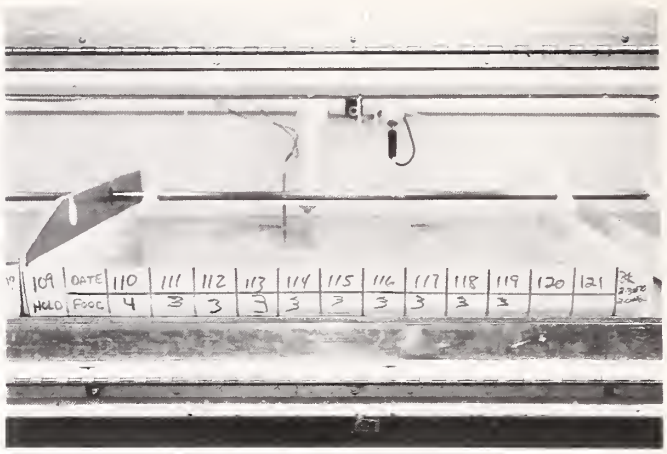

Figure 32. Pupal rearing pan with proper label listing the current and consecutive Julian dates and the preparer's initials

3. Monitor the temperatures of the water in the pupal rearing pans daily, record these values on form D112 *Colonies—Physical Parameters* (app. D), remove the scum that has formed at the water surface along and on the surface of the islands and along the edges of the pan with an aquarium algae scraper daily, and use a moistened sponge and a small radial-tip test tube brush to remove the scum on the paddles as it accumulates.

Collection of pupae from pupal rearing pans

Caution: Make sure to use the scraper before doing step 4 or else the scum along the edges of the pan and islands will be collected with the pupae.

4. Place a 5-inch-wide flotation screen on each 4-inch-wide island that contains pupae, press down so that each island is submerged, add more DI water (if necessary) to completely submerge the islands, and wait approximately 30 min.

Note: The pupae will float to the water surface and will usually congregate along the edges of the pan or will float freely with the circulating water; otherwise, the pupae will drown after a few hours.

5. Connect the flexible tubing between the 2,000-ml vacuum flask on the movable 6-ft-high floor stand (½-inch threaded pipe attached to a chair base with casters) and the vacuum pump, and turn on the pump.

Note: The components of the pupal collecting system are shown in figure 33.

6. Use the pupal collecting system to aspirate the pupae from the holding pans (fig. 34).

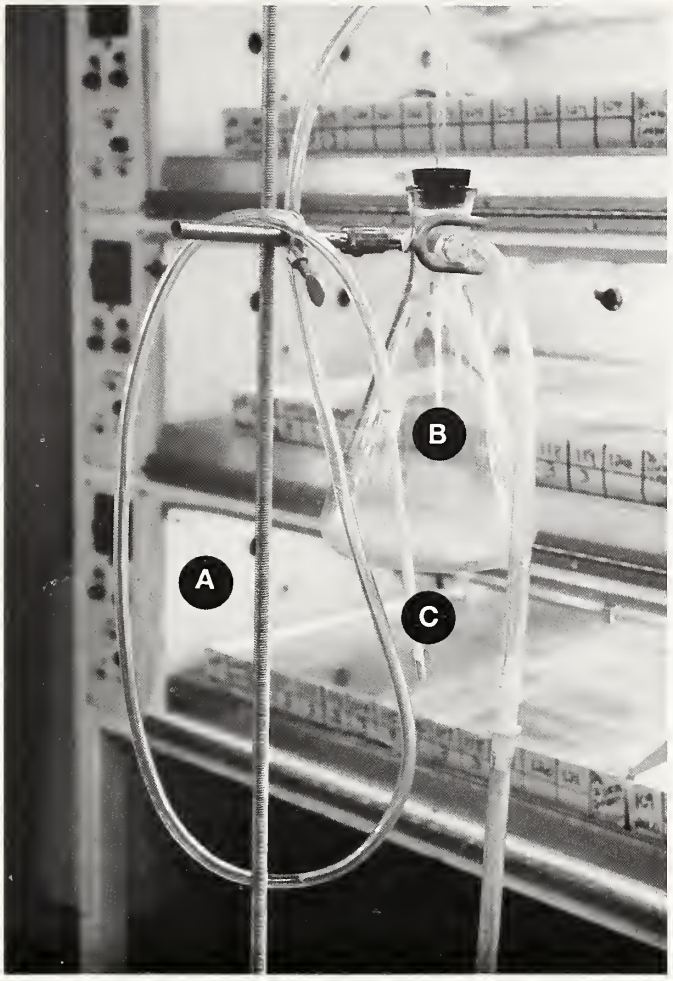

Figure 33. Components of the pupal collecting system. *A*, Floor stand. *B*, Vacuum flask. *C*, Aspirator.

7. After the flask is full, empty the contents into a No. 140 sieve, reconnect the pupal collecting system, and collect any remaining pupae (if necessary).

Caution: Do not allow the flask to overflow because the pump may be damaged.

8. Transfer the pupae from the sieve to a round enamel pan, and use the spray hose to create a swirling movement of the water in the pan.

Note: A few mature larvae will be unintentionally collected during the aspiration of pupae; the swirling motion of the water forces the larvae to congregate at the bottom and near the center of the pan for easy removal.

9. Use a human rectal bulb to collect the larvae on the bottom near the center of the pan, place the larvae collections (which include a few pupae) into another round pan, remove the pupae, and transfer the larvae to the recently prepared pupal rearing pans.

Figure 34. Pupae being aspirated from a pupal rearing pan

10. Transfer the pupae in the pan to a beaker, remove the screens from the pupal rearing pans, and refloat the islands at the water surface.

Caution: Make sure the screens are removed and all islands are floating properly or else the remaining mature larvae will drown.

Note: The process of collecting pupae is known as picking.

11. Clean the screens and the pieces of the pupal collecting system using warm water, and air dry the equipment.

Note: The pupae should be collected every Monday, Wednesday, and Friday and every Saturday or Sunday from all 12 pupal rearing pans to provide a continuous supply of insects

12. After the 14-day period for maintaining each pair of pupal rearing pans, remove the four islands, kill the remaining mature larvae in the islands with hot water, and discard the material in an autoclavable waste bag.

13. Remove and clean the paddles, metal bars, thermometer (if in use), and pans with a sponge and warm water; in addition, clean the empty shelf spaces, stirring rods, water-filling devices, float units, and thermistors (if in use) with a moistened sponge.

14. Return the two clean pupal rearing pans and the accessories to their proper locations in the small rearing rack, prepare the pans (see steps 1–3 in *Production of Pupae*), and record the pan numbers on form D116 *Colonies—Larval Production and Immature Use* (app. D).

Volumetric measurement of pupae collected

15. Measure the volume of pupae collected by the same procedure used to measure the volume of mature larvae collected (see steps 45–49 in *Production of Larvae*); however, use a pupal measuring tube (app. F explains how to make this tube).

16. Disassemble the pieces of the pupal measuring system, clean them with warm water, and air dry the pieces.

Preparation of emergence containers

17. Fill an O/E container with sterile cotton, soak the material with DI water, smooth out the surface with your fingers, and drain the excess water.

Note: The moist substrate should be approximately ⅜ inch thick. The following guidelines should be used to prepare the proper number of O/E containers:

Pupae collected (ml)	Number of O/E containers needed
<4.0	1
4.0–8.0	2
8.0–12.0	3
12.0–16.0	4
16.0–20.0	5

18. Place a finger over the screened end of the pupal measuring tube, pour a small amount of DI water from a 250-ml beaker into the tube, cover the other end, and shake the tube to loosen the compacted pupae.

19. Pour approximately an equal amount of pupae into each O/E container, use a glass eye dropper to spread the pupae evenly across the cotton, set the containers aside for approximately 5 min, and record the approximate number of pupae added to each container on form D111 *Colonies—Adult Production* (app. D).

Note: The 5-min period allows the pupae to embed into the cotton material; this is necessary for proper emergence of the adults.

20. Invert the O/E container to drain off the excess water.

21. Insert the O/E container into an adult holding cage (see step 6 in *Production of Adults*).

Miscellaneous

Recording information on data collection forms and flow charts

1. Record the starting and ending time(s) and the initials of the persons who conducted insect production and maintenance; record the name of insectary supply items, and the initials of the persons who prepared each item on form D121 *Colonies—Production Costs* (app. D).

2. If shipments of eggs, larvae, pupae, or adults to authorized laboratories are required, record this information on form D122 *Colonies—Shipments to Other Laboratories* (app. D).

3. Each day complete the checklists on flow charts FC101 *Colony Operations*, FC102 *Scheduled Colony Use*, and FC103 *Colony Room Maintenance* (app. D).

4. Check the form *Request for Insects from Insect Culture Production* (app. D) daily to determine which insect stage is being scheduled for in-house use or for shipment to an authorized laboratory, and respond accordingly.

Summary

The weekly work schedule of the procedures for the large-scale rearing of *C. variipennis* is summarized in table 1.

QUALITY CONTROL

The quality of mass-reared insects is generally defined and measured in terms of how well the insect population functions in its intended role either in the field or laboratory (Huettel 1976). Boller and Chambers (1977) defined quality control as the mechanism to provide and coordinate a production system which ensures that the operation will produce adequate numbers of an optimum quality at minimum product costs. In addition, overall quality can be divided into five major components covering adaptability, motility, orientation to habitat, sexual activities (for example, courtship and mating), and reproduction. Chambers (1977) stated that variables that assess quality are usually measured to (1) monitor the quality of the rearing process, (2) ensure continuity in production facilities, and (3) assess insect development, reproduction, and survival. These variables include egg viability; density and yield of immature stages; survival, size, and yield of adults; density, sex ratio, and age of adults; life history measurements; texture, composition, and microbial load of diet; and environmental conditions.

The evaluation of laboratory-reared *C. variipennis* is difficult and requires objective testing. Quality control criteria and protocols have been established at ABADRL to assess the quality and the continuity of insect production. Quality evaluation of *C. variipennis* is determined by reviewing major production records and performing measurements on the insects produced. Production data are constantly reviewed to ensure consistency of production and to allow for the early detection of problems. Data used routinely (weekly or biweekly) include (1) adult dry weight and adult wing length, (2) gross egg production, (3) gross third- and fourth-instar larval production, (4) gross pupal production, (5) insect feeding schedules, and (6) temperatures. The text that follows explains how to measure adult dry weight and wing length. Presently, systems analysis and automated data processing technology provide efficient mechanisms for gathering, storing, retrieving, analyzing, and sharing data associated with the production of *C. variipennis* (Akey et al. 1984). Coordination of these data from production records aids in production forecasts and, thereby, provides a valuable management tool.

Table 1. Weekly work schedule for the large-scale rearing of *Culicoides variipennis*

Insect production and maintenance	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Production of adults							
Adult emergence*	Crop	Crop	Crop	Crop	Crop	Crop	Crop
Maintenance of adults	Burp	Burp	Burp	Burp	Burp	Burp	Burp
Production of eggs							
Blood feeding†		Blood feed		Blood feed		Blood feed	
Collection of eggs	Collect from B cages	Place O/E containers in C cages	Collect from C cages		Place O/E containers in A cages	Collect from A cages	Place O/E containers in B cages
Production of larvae							
Preparation and maintenance of egg hatch pans #21–23‡				Prepare and maintain pan #21	Prepare and maintain pan #22	Prepare and maintain pan #23	
Preparation and maintenance of larval rearing pans #1–18§		Prepare and maintain pans #1–6	Prepare and maintain pans #7–12	Prepare and maintain pans # 13–18. Add ½ tsp of "J" media to pans #1–6	Add ½ tsp of "J" media to pans # 7–12	Add ½ tsp of "J" media to pans #13–18	
Transfer of egg hatch islands to larval rearing pans‖				Transfer 6 islands from pan #21 to pans #1–6	Transfer 6 islands from pan #22 to pans #7–12	Transfer 6 islands from pan #23 to pans #13–18	
Removal of egg hatch islands from larval rearing pans					Remove islands from pans #1–6	Remove islands from pans #7–12	Remove islands from pans #13–18
Collection and measurement of mature larvae¶		Collect and measure larva from pans #1–6 and transfer to pans #24 and 25 or to pans #30 and 31	Collect and measure larvae from pans #7–12 and transfer to pans #27 and 28 or to pans #33 and 34	Collect and measure larvae from pans #13–18 and transfer to pans #26 and 29 or to pans #32 and 35			
Production of pupae							
Preparation and maintenance of pupal rearing pans #24–35**	Prepare and maintain pans #24 and 25 or pans #30 and 31	Prepare and maintain pans #27 and 28 or pans # 33 and 34					Prepare and maintain pans #26 and 29 or pans #32 and 35
Collection and measurement of pupae	Pick today or Saturday	Pick		Pick		Pick	Pick today or Sunday

*Use 3 vials of DI water.

†Blood feed all adults in adult holding cages that have been cropped and that are at least 24-hours old. Use the youngest adults first and discard the older ones if they are not needed. If available, always save one cage, (for example, 24- to 48-hour-old adults) for emergency or unscheduled research use. Use up to 5 ml of adults per cone used for blood feeding. Use two vials of 10 percent sucrose solution and one vial of DI water.

‡Add 6 ml of bacterial inoculum, 2 ml of nutrient broth fluid concentrate, 2 level tsp of "Kalf" media, and approximately 60,000 eggs on the first day of preparation. Add the appropriate amount of nutrient broth daily for maintenance (see egg hatch label).

§Add 6 ml of bacterial inoculum, 2 ml of nutrient broth fluid concentrate, and 2 level tsp of "Kalf" media on the first day of preparation. Add the appropriate amount of nutrient broth daily for maintenance (see larval rearing label.)

‖Collect larvae from the pan water with a No. 170 sieve, and place the larvae in one of the larval rearing pans.

¶Place up to 5 ml (that is, 10,000) No. 70 sieve-size larvae into each of two pupal rearing pans. If there is an excess of larvae, place these larvae into one or more recently prepared pupal rearing pans that did not reach the optimal level; otherwise, discard the excess larvae. If there are less than 10,000 No. 70 sieve-size larvae per pan, the difference can be made up by adding 3,000 No. 80 sieve-size larvae per 1 ml. Eight thousand No. 140 sieve-size larvae per 1 ml can also be used to supplement the No. 70 sieve-size larvae if there are not enough No. 80 sieve-size larvae to increase the total to 10,000 larvae per pan.

**Add 6 ml of bacterial inoculum, 4 ml of nutrient broth fluid concentrate, and 2 level tsp of "Kalf" media on the first day of preparation. Add the appropriate amount of nutrient broth daily for maintenance (see pupal rearing label).

Measurement of Adult Wing Lengths and Adult Dry Weights

1. During a pupal collecting day (Monday, Wednesday, Friday, and Saturday or Sunday), place 200 pupae in an emergence container (see steps 17–21 in *Production of Pupae*), prepare an adult holding cage (see steps 1–7 in *Production of Adults*), and maintain the emerging adults for three days (see steps 7–21 in *Production of Adults*).

2. During this 3-day period, crop the cages to obtain 24- to 48-hr-old adults, anesthetize the adults with CO_2, and immediately place the adults in the freezer.

3. Use hot water to kill the remaining pupae in the cotton substrate, and discard the material in an autoclavable waste bag.

4. Carefully remove one complete wing from 24 female adults with a pair of forceps, place three wings on each of eight microscope slides, and label the wings 1–24; place the adults in individual glass vials, and label the vials 1–24 to match the wing numbers.

5. Set a stereoscopic microscope at X 40 magnification, and calibrate it with a micrometer disk to determine the number of drum units per 1-mm division; record this value on form *Quality Control: Adult Wing Length/Adult Dry Weight* (app. D).

6. Exchange the right ocular eyepiece with the ocular micrometer, and measure the length of the wing from the basal arculus to the tip. Record this value on the same form. Use the conversion formula (corrected wing length value = wing length/number of drum units) to determine the corrected wing length value, record this value on the same form, and analyze the data.

7. Place the 24 vials in the oven for 2 hr at 75 °C.

8. Use the electronic microbalance to weigh each dried adult (minus one wing). Record the dry weight values with their corresponding wing length values on the same form, and analyze the data.

Note: The adults may be stored indefinitely in the freezer to delay weighing them until a more convenient time.

Evaluation of New Insect Rearing Materials

The only safe approach for evaluating new insect rearing materials, such as dietary ingredients, disposable rearing materials, and small insectary equipment, prior to their general use is by conducting small-scale investigations separate from the standard rearing program. The following procedures should be followed when evaluating new materials:

1. Prepare and maintain two egg hatch pans in the quality control incubator (see steps 1–10 in *Production of Larvae*).

Note: One egg hatch pan should be maintained to evaluate the new insect rearing material, and the other pan should be maintained as a control for the current rearing materials.

2. Two days before transferring larvae from the egg hatch pans to the larval rearing pans, prepare and maintain two larval rearing pans in the incubator (see steps 11–14 in *Production of Larvae*).

Note: One larval rearing pan should be maintained to evaluate the new insect rearing material, and the other pan should be maintained as a control for the current rearing materials.

3. After 7 days of maintaining the egg hatch pans, transfer one 2-inch-wide Dacron island with small larvae from each egg hatch pan to the appropriate larval rearing pans (see steps 15–28 in *Production of Larvae*), use hot water to kill the larvae in the remaining four islands, and discard the material in an autoclavable waste bag.

4. Two days before transferring mature larvae from the larval rearing pans to the pupal rearing pans, prepare and maintain two pupal rearing pans in the incubator (see steps 1–3 in *Production of Pupae*).

5. After 7 days of maintaining the larval rearing pans, transfer the islands containing mature larvae from the larval rearing pans to the appropriate pupal rearing pans. Instead of putting a pair of islands in each pan, just put one island in each pan.

Note: One pupal rearing pan should be maintained to evaluate the new insect rearing material, and the other pan should be maintained as a control for the current rearing materials.

6. Collect pupae from the pupal rearing pans (see steps 4–13 in *Production of Pupae*).

7. Prepare two O/E containers (see steps 17–21 in *Production of Pupae*), prepare two adult holding cages (see steps 1–6 in *Production of Adults*), and maintain the emergence cages for 3 days (see steps 7–21 in *Production of Adults*).

8. During the 3-day period, collect approximately 200 24- to 48-hr-old adults to determine 24 wing length and dry weight values (see the procedures for measuring adult wing length and adult dry weight on the previous page); record these values on form *Quality Control: Adult Wing Length/Adult Dry Weight* (app. D), and then analyze the data.

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APPENDIX A. INSECT SECURITY

Extreme precautions must be taken to prevent the escape of the biting midge and to prevent the introduction of any extraneous insects into the insect rearing facility. A number of physical barriers must be established. These barriers include (1) a security vestibule consisting of two screened doorways located at the entrance of each rearing room in which a woven synthetic fabric (52 by 52 threads per square inch) prevents the movement of insects in either direction, (2) the sealing or containment of all openings and room penetrations (for example, electrical and plumbing lines; electrical fixtures and outlets; doorjambs; floor, wall, and ceiling fixtures; central air vents; and exhaust pipes), and (3) the placement of strategically located insect light traps (fig. 35) to collect escaped adult midges and certain extraneous flying insects entering the facility. Furthermore, the following security procedures must be strictly adhered to:

1. The horizontal and vertical zippers on the screened doorways must remain closed at all times to prevent the entry or exit of all insects.

2. Employees must wear the laboratory coats assigned to each rearing room in that room only. This will prevent the possible transfer of insects between each room via lab coats.

3. Hot water must be used to kill any eggs, larvae, pupae, or adults discarded in the sink to prevent the possible escape of these insects through the waste drainage system and into the local environment.

4. All disposable rearing materials (for example, Dacron islands, vial wicks, depleted adult holding cages, depleted egg holding containers, and cotton substrates used for adult emergence or oviposition) must be put into autoclavable waste bags, properly autoclaved, and disposed to prevent the possible release of any insect stage through the waste disposal system and into the local environment.

5. Any live insect stage leaving the insect rearing facility for use in research must not be permitted back into the facility. This procedure will prevent the viral, microbial, or insecticidal contamination of the insect colony.

6. An inspection for ants, beetles, and other insects must be conducted every day to prevent the contamination or destruction of the insect colony or diet ingredients.

Figure 35. Insect light trap

APPENDIX B.
OBTAINING THE BITING MIDGE

The insect rearing program at the USDA-ARS ABADRL occasionally maintains production levels of *C. variipennis* greater than actual needs for ongoing research. Eggs, larvae, pupae, or adults are available without charge upon receipt of reasonable requests from authorized educational, governmental, or industrial laboratories. However, the importation or subsequent receipt of this vector species of animal diseases is subject to regulations of USDA's Animal and Plant Health Inspection Service (APHIS) (9 CFR 94 and 9 CFR 122). A request for *C. variipennis* must be provided at least 2 wk in advance; both written and oral requests are accepted. Eggs constitute the most readily available and convenient insect stage for shipment. Shipment is scheduled for 24-hr delivery and is made early in the day and early in the week to avoid delays in transit. The shipping container must be returned at the requester's expense.

Those investigators interested in establishing a laboratory colony or receiving a specific insect stage of *C. variipennis* for research use are advised to discuss special instructions on the small-scale rearing of this species or to arrange for shipping by contacting ABADRL at the following address and phone numbers:

Arthropod-borne Animal Diseases Research Laboratory
USDA–ARS
P.O. Box 3965, University Station
Laramie, WY 82071–3965

Commercial (307) 766–3600
FAX (307) 766–3500.

Before ABADRL will ship insects to requesters, they must obtain a permit authorizing the importation or receipt of regulated materials and specifying conditions under which the vector is to be shipped, handled, and used. These permits are issued annually by APHIS. Allow at least 3 wk to obtain a permit. Permits and additional information on the importation and interstate shipment of *C. variipennis* and other related materials may be obtained by contacting

Import-Export & Emergency Planning
USDA–APHIS, Veterinary Services
Room 810, Federal Building
6505 Belcrest Road
Hyattsville, MD 20782

Commercial (301) 436–8084
FAX (301) 436–8226.

APPENDIX C.
USES AND SOURCES OF EQUIPMENT

Various types of equipment are used in the insect rearing program to mechanize routines, thereby reducing the amount of labor required. Some of the equipment used at ABADRL has been specifically designed and fabricated at ABADRL; however, some of it is commercial equipment with or without modifications. The uses and sources of the major pieces of equipment that have proved suitable for the large-scale rearing of *C. variipennis* are listed by production categories. This listing is not meant to imply that other products are unsuitable—there may be several others that are sufficient or better.

Uses of Equipment

Adult production

Environmental growth incubator: A commercial unit used to maintain the correct temperature (26.5 °C ± 1 °C), lighting (a photoperiod of 13:11 (L:D) hr), and humidity (40–60 percent relative humidity (RH)) to sustain adults for varying holding periods.

Relative humidity and temperature recorder: A commercial unit used to measure and record temperature and RH for a 7-day cycle within the environmental growth incubator.

Egg production

Artificial apparatus for blood feeding: A custom-made commercial unit used to allow adults to feed on temperature-controlled (36.5 °C) and uniformly mixed sheep blood through a reinforced silicone membrane.

Freezer: A commercial unit used to store adults to be used for quality control measurements.

Refrigerator: A commercial unit used to maintain the correct temperature (approximately 7 °C) to prolong the viability of eggs for up to 30 days and to provide maximum shelf life for dietary formulations (bacterial inoculum, nutrient broth fluid concentrate, sheep blood, and 10 percent sucrose solution).

Larval production

Insect rearing racks: Custom-made units used to maintain the correct temperature (26.5 °C), lighting (a photoperiod of 13:11 (L:D) hr), water level, and water movement in the insect rearing pans for the immature insect stages. The small rearing rack consists of 15 spaces for insect rearing pans, and the large rack contains 20 spaces.

Larval collecting system: A custom-made system used to collect third- and fourth-instar larvae from the insect rearing pans.

Larval measuring system: A custom-made system used to measure the approximate number of larvae collected via the larval collecting system.

Pupal production

Insect rearing rack: See definition in *Larval Production* in this appendix.

Pupal collecting system: A custom-made system used to collect pupae from the insect rearing pans.

Pupal measuring system: A custom-made system used to measure the approximate number of pupae collected via the pupal collecting system.

Dietary preparation

Bacteriological incubator: A commercial unit used to maintain the correct temperature (37 °C) for incubating bottles of bacterial inoculum.

Hand mill: A commercial unit used to grind a coarse dietary ingredient (high-protein supplement) into a fine powder.

Hot plate/stirrer: A commercial unit used to dissolve a dietary ingredient (nutrient broth medium) in heated DI water.

Portable electronic balance: A commercial unit used to weigh dietary ingredients (albumin, alfalfa, brain-heart infusion medium, high-protein supplement, nutrient broth medium, and yeast extract medium).

Refrigerator: See definition in *Egg Production* in this appendix.

Quality control

Data base management system: A personal computer used to gather, store, retrieve, analyze, and communicate assessment of data associated with insect production.

Electronic micro balance: A commercial unit used to weigh dried adults.

Oven: A commercial unit used to maintain the correct temperature (75 °C) for drying adults for dry weight measurements.

Quality control incubator: A custom-made commercial unit used to maintain the correct temperature (26.5 °C ± 1 °C), lighting (a photoperiod of 13:11 (L:D) hr), and water movement in the insect rearing pans during testing of new insect rearing materials (dietary ingredients, Dacron islands, and small insectary equipment). The incubator is used as part of the quality control program to test materials before they are integrated into the standard rearing procedures.

Stereoscopic microscope: A commercial unit used to measure the wing lengths of adults.

Miscellaneous

Autoclave: A commercial unit used to sterilize dietary formulations (nutrient broth fluid concentrate and bacterial inoculum) and small insectary equipment (rearing pan screens and

weights, O/E containers, and water-jacketed glass feeders) for 20 min at 15 lb of pressure and at 121 °C.

Carbon dioxide system: A commercial system of medical-grade carbon dioxide used to anesthetize adults before and after blood feeding and used to kill adults prior to discarding them in a sink.

Deionized water system: A commercial system used to provide high-purity water for the proper maintenance of insects in all stages.

Heating chamber and temperature-regulating device: A custom-made system used to fabricate paddles for the dispersal of bacterial scum in the aquatic rearing media.

Insect light traps: Commercial units strategically placed throughout the insect rearing facility to collect escaped adults of *C. variipennis* and certain extraneous flying insects entering the facility.

Sources of Equipment

The listed sources are where ABADRL has been able to locate equipment and are not provided as a recommendation over any others. The addresses provided in this list were current at the time of publication but may have since changed.

Artificial blood feeder: See Hunt and McKinnon (1990)

Autoclave, model STM–E: Market Forge Co., 35 Garvey Street, Everett, MA 02149

Bacteriological incubator, model 200A: Blue M, 2218 W. 138th, Blue Island, IL 60406

Carbon dioxide system: Locally available at a welding supply company

Deionized water system: Locally available at a water conditioning company

Electronic micro balance, model Sartorius S4: Brinkman Instruments, Inc., Cartique Road, Westbury, NY 11590

Environmental growth incubator, model I–35VLD with options C1, F, G, and N: Percival Manufacturing Co., P.O. Box 249, Boone, IA 50036

Freezer, model Kenmore 198.8261685: Sears, Roebuck, and Co., Dept. 698/731A, Sears Tower, Chicago, IL 60684

Hand mill, model Corona Mill: R & R Mill Co., Inc., 45 W. 1st Northy Street, Smithfield, UT 84335

Hot plate/stirrer, model PC–101: Corning Glass Works, Corning, NY 14830

Insect light traps, model Miniature Black Light Traps: John W. Hock Co., P.O. Box 12852, Gainesville, FL 32604

Oven, model OV–18A: Blue M, Blue Island, IL 60406

Portable electronic balance, model Sartorius 1002MP9: Brinkman Instruments, Inc., Cartique Road, Westbury, NY 11590

Quality control incubator, model 3920: Forma Scientific, Inc., Marietta, OH 45750

Refrigerator, model VWR 2010: VWR Scientific, Inc., P.O. Box 39396, Denver, CO 80239

Relative humidity and temperature recorder, model 22–7078: Bacharach, Inc., 625 Alpha Drive, Pittsburgh, PA 15238

Stereoscopic microscope, model Zeiss Stereozoom: E. Licht Co., 3255 S. Acoma Street, Englewood, CO 80110

APPENDIX D. DATA COLLECTION FORMS AND FLOW CHARTS

The filled-out forms in this section are provided as samples. Blank forms are provided at the end of this appendix for the reader to use and duplicate. The following codes are used in the samples:

Adult Nutrition Codes
00—Blank (that is, no nutrition)
01—DI water
02—10 percent sucrose solution

Counting Method Codes
1—Volume (ml)
2—Estimate
3—Actual count
4—Cropped
5—Pupae split
6—Estimate split

Stadium Codes
1—Eggs
2—Larvae
3—Pupae, female
4—Pupae, male
5—Pupae, mixed
6—Adults, female
7—Adults, male
8—Adults, mixed

Usage Codes
01—Colony (for example, A = Monday, B = Wednesday, and C = Friday)
02—Quality control
03—Other authorized laboratory
04—15 Assigned to various researchers at ABADRL
NF—No flies
NN—Not needed (for example, discarded)

SHEET NO. ____________

COLONIES – ADULT PRODUCTION
D 111 *(Entomology)*

$b̸ ________ b̸ ________ b̸ ________ b̸ ________ b̸ ________ R
YEAR *(2)* — ROOM *(2)* — INCUBATOR *(2)* — COLONY *(2)* — FOOD TYPE (A) in [4] *(2)*

POP. [1]	[2]	EMERGENCE TIME [3]				ADULT NUTRITION [4]							PUPAL PRODUCTION [5]										USE [6]				
		Start		End		(B)			(C)				(A)			(B)			(C)				(A)		(B)		
Cage No.	GENER.	Day	Hour *(nearest)*	Day	Hour *(nearest)*	Day	Hour *(nearest)*	Food Type	Day	Hour *(nearest)*	Food Type		Source Day	CM	No.	Source Day	CM	No.	Source Day	CM	No.		Day	Who	Day	Who	
4	*2*	*3*	*4*	*3*	*4*	*3*	*4*	*2*	*3*	*4*	*2*		*3*	*1*	*4*	*3*	*1*	*4*	*3*	*1*	*4*		*3*	*2*	*3*	*2*	
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NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D111)**

b̸, |, or | = Space *(hit space bar)*
R = Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

Form WR-704 (12/79)

USDA-SEA

COLONIES – PHYSICAL PARAMETERS

D112 *(Entomology)*

SHEET NO. 5

$ ␣ 93 ␣ 8 ␣ ␣ R
YEAR (2) ROOM (2) ROOM (2) ROOM (2)

[1]	LARVAL PAN LOCATION AND TEMPERATURE °C [2]																HOLDING CONDITIONS [3]													
	(A)		(B)		(C)		(D)		(E)		(F)		(G)		(H)		(A)					(B)					(C)				
																		Temp. °C		RH			Temp. °C		RH			Temp. °C		RH	
DAY	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Inc. No.	High	Low	H	L	Inc. No.	High	Low	H	L	Inc. No.	High	Low	H	L
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	2	2	2	3	3	2	2	2	3	3	2	2
041	3	260	7	260	11	260	15	265	19	260	21	275	22	275	23	265	63	270	245	54	51		090	080							
041	25	265	28	260	31	270	34	265																							
042	3	260	7	260	11	260	15	265	19	260	21	280	22	275	23	265	63	270	240	58	55		090	075							
042	25	265	28	260	31	270	34	265																							
043	3	260	7	265	11	260	15	270	19	260	21	270	22	275	23	265	63	265	245	60	58		080	070							
043	25	265	28	265	31	270	34	265																							

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D112)**

␣, |, or ¦ = Space *(hit space bar)*
R = Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

Form ARS-720 (1/86)
(Prev. edition 12/79 may be used)

USDA-ARS

COLONIES – PUPAE PRODUCTION
D113 *(Entomology)*

$b̸ 93 (YEAR (2)) b̸ 8 (ROOM (2)) b̸ AA (COLONY (2)) R

[1] DAY *3*	[2] PUPAE *(no./ml)* *3*		[1] DAY *3*	[2] PUPAE *(no./ml)* *3*		[1] DAY *3*	[2] PUPAE *(no./ml)* *3*	
099	1460	R			R			R
125	1420	R			R			R
236	1370	R			R			R
301	1400	R			R			R
360	1390	R			R			R
		R			R			R
		R			R			R
		R			R			R
		R			R			R
		R			R			R
		R			R			R
		R			R			R
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		R			R			R
		R			R			R

For Help key in:
LIST HELP(D113)

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

Form WR-705 (12/79) USDA-SEA

SHEET NO. 2

D113 and D122 are *NOT* related.

NOTE: Italic numerals indicate maximum number of characters to be used.

b̸, |, or ¦ = Space *(hit space bar)*
R= Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

COLONIES – SHIPMENTS TO OTHER LABORATORIES
D122 *(Entomology)*

$b̸ 93 (YEAR (2)) b̸ AA (COLONY (2)) R

[1] DAY	[2] NAME OF RECIPIENT LAB	HOW [3]			COST to ABADRL [4]			
		Stadium	Counting Method	No.	Shipping Method	Cost $	Cost ¢	
3	*8*	*1*	*1*	*4*	*8*	*3*	*2*	
051	CSU	6	2	0010	Vehicle			R
090	LSU	1	2	0700	Fed Exp			R
239	LSU	1	2	0600	Fed Exp			R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R
								R

For Help key in:
LIST HELP(D 122)

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

U.S. Government Printi ice·1980·682·947/319

COLONIES – EGG PRODUCTION

D114 *(Entomology)*

SHEET NO. 3

$ ␣ 93 ␣ 8 ␣ AA R

YEAR (2) ROOM (2) COLONY (2)

[1] DAY	[2] CAGES BF		EGG PRODUCTION [3] (A)				(B)				(C)				EGG USE [4] (A)				(B)				(C)				HATCH [5]		[6] COLONY FLY USE	
	New	Old	Day	CM	No. (K)	Inc. No.	Day	CM	No. (K)	Inc. No.	Day	CM	No. (K)	Inc. No.	Day	CM	No. (K)	Who	Day	CM	No. (K)	Who	Day	CM	No. (K)	Who	Number Counted	Number Hatched	Day	Who
3	1	2	3	1	4	2	3	1	4	2	3	1	4	2	3	1	4	2	3	1	4	2	3	1	4	2	3	3	3	2
048	A		052	2	0300	2									056	2	0300	9											055	A
050	B		054	2	0400	2									056	2	0400	9											055	A
052	C		056	2	0450	2	057	2	0050						058	2	0500	22											057	B
055	A	A/B	059	2	1000	2									059	2	0700	23	064	2	0300	21							059	C
057	B	C	061	2	0900	2									064	2	0400	21	065	2	0500	22							061	NN
059	C	A	063	2	0850	2									065	2	0200	22	066	2	0650	23							063	NN
061	A		065	2	0900	2																								

R

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D114)**

␣, |, or | = Space *(hit space bar)*

R = Return *(hit RETURN key)*

| = Beginning/Ending of field *(do not space)*

	1	2	3	4	5
K					
LF					
V					

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Form WR-706 (12/79)

USDA-SEA

U.S. Government Print' Office:1980-682-942/314

COLONIES — LARVAL PRODUCTION AND IMMATURE USE
D116 *(Entomology)*

SHEET NO. 4

FOOD TYPE ______

$ b̸ 93 b̸ 8 b̸ AA R
YEAR *(2)* ROOM *(2)* COLONY *(2)*

[1]	LARVAL REARING PAN NUMBERS												HOLDING PANS												[7] LARVAL PANS — Nonstandard Nutrition										[8] USE				
DAY *3*	[2] DISCARD					[3] To Holding *2*	[4] SETUP					[5] *Setup* Pan No. *2*	Nutrition [6]																										
	(A) *2*	(B) *2*	(C) *2*	(D) *2*	(E) *2*		(A) *2*	(B) *2*	(C) *2*	(D) *2*	(E) *2*		(A) Pan No. *2*	Food *2*	(B) Pan No. *2*	Food *2*	(C) Pan No. *2*	Food *2*	(D) Pan No. *2*	Food *2*	(E) Pan No. *2*	Food *2*	(F) Pan No. *2*	Food *2*	(A) Pan No. *2*	Food *2*	(B) Pan No. *2*	Food *2*	(C) Pan No. *2*	Food *2*	(D) Pan No. *2*	Food *2*	(E) Pan No. *2*	Food *2*	Who *2*	Stadium *1*	C M *1*	No. *4*	
041	7	8	9	10	11	R 33	7	8	9	10	11	R	24	3	25	3	26	3	27	3	28	3	29	3	R 21	2	22	2	23	2					R				R
041	12					R 34	12					R	30	3	31	3	32	4	33	3	34	3	35	4	R										R				R
042	13	14	15	16	17	R 32	13	14	15	16	17	R	24	3	25	3	26	4	27	3	28	3	29	3	R 22	2	23	2							R 2	5	3	0100	R
042	18	21				R 35	18	21				R	30	3	31	3	32	3	33	3	34	3	35	3	R										R 10	5	3	0050	R
043	22					R	22					R	24	3	25	3	26	3	27	3	28	3	29	3	R 21	2	23	2							R 10	5	2	0050	R
043						R						R	30	3	31	3	32	3	33	3	34	3	35	3	R										R				R
044	23					R	23					R	24	3	25	3	26	3	27	3	28	3	29	3	R 21	2	22	2							R				R
044						R						R	30	3	31	3	32	3	33	3	34	3	35	3	R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R
						R						R													R										R				R

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D116)**

b̸, |, or | = Space *(hit space bar)*
R = Return *(hit RETURN key)*
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	1	2	3	4	5
K					
LF					
V					

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Form WR-709 (12/79)

USDA-SEA

U.S. Governmen' ›ting Office-1980-682-944/31‹

COLONIES -- PRODUCTION COSTS
D121 *(Entomology)*

SHEET NO. 1

$␣ 93 ␣ 8 ␣ AA ␣
YEAR (2) ROOM (2) COLONY (2)

[1] DAY	PERSONNEL USE [2] Initials		Time (exact minutes)		T or H	[3] MAINTENANCE and SUPPLY MAKEUP and USE					[4] MAINTENANCE and SUPPLY COSTS				
	Scheduled	Done by	Start	End		Name Item	No. of	Initials	Time (exact minutes) Start	End	Name Item	No. of	Cost $	¢	
3	2	2	4	4	1	8	3	2	4	4	8	3	4	2	
001	JK	JK	0630	1200		R					R				R
002	JK	JK	0630	1300		R sugar water	001	JK	start	use	R				R
003	JK	JK	0700	1200		R					R				R
004	GH	GH	0700	1400		R nutrient broth	001	JK	1000	1245	R				R
005	JK	JK	0630	1100		R					R				R
006	JK	GH	0630	1200		R "K" media	004	JK	1100	1130	R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R
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						R					R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R
						R					R				R

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D121)**

␣, | , or | = Space *(hit space bar)*
R = Return *(hit RETURN key)*
| | = Beginning/Ending of field *(do not space)*

Enter R only at completion of same source day after each field.

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

Form WR - 708 (12/79)

USDA-SEA

U.S. Government Printing Office: 1980-682-943/315

93 YEAR | AA COLONY | 8 ROOM

COLONIES-- LARVAL PRODUCTION

Date	Total larvae ml (Each sieve)	Total larvae ml (All sieves)	Larvae: ml and use			Excess larvae: pan # or discard
			Use			
			Pan #	ml	Pan #	
005	10.4	6.3 #70 3.8 #80	24	2.9 #70 1.9 #80	25	Discard
006	11.2	6.6 #70 5.5 #80	27	3.0 #70 1.0 #80	28	Discard
007	5.5	5.5 #80	26	2.7 #80	29	Discard
012	8.6	5.6 #70 2.5 #80	30	2.8 #70 1.7 #80	31	Discard
013	16.0	4.6 #70 6.0 #80	33	2.3 #70 1.5 #80	34	Few #80 on 24-29
014	14.0	6.6 #70 5.5 #80	30	3.3 #70 0.7 #80	35	Discard

93 Year | AA Colony | 8 Room

Sheet No. 1

COLONY OPERATIONS - CULICOIDES
FC 101 (Entomology)

Action		Data entry	Scheduled	Julian dates M 001	Tu 002	W 003	Th 004	F 005	Sa 006	Su 007
COMPLETED PHYSICAL PARAMETERS		D112	Daily	X						
Larval pans	Water added		Daily	X						
	Scum (film) broken up		Daily	X						
	Discarded	D116	See schedule	X						
	Setup	D116	See schedule	X						
Eggs placed on egg hatch pans		D114	W, Th, F	-						
Egg hatch pans to larval rearing pans		D117	W, Th, F	-						
Larval rearing pans to pupal rearing		D117	M, Tu, W	X						
Pupae recovered		D111	M, W, F, Sa, or Su	X						
Food added to egg hatch, larval rearing, & pupal pans	Bacterial inoculum		At setup	X						
	"Kalf" medium		At setup	X						
	"J" medium		W, Th, F	-						
	Nutrient broth fluid concentrate	D116	Daily	X						
COMPLETED LARVAL PRODUCTION		D116	Daily	X						
Colony cages (A-C) & scheduling for	Oviposition dish placed	D114	M, Th, Sa	X						
	Eggs recorded	D114	Tu, F, Su	-						
	Egg use recorded	D114	W, Th, F	-						
	Stored eggs moistened		As needed	-						
	Cages blood fed	D114	M, W, F	X						
	Food (2 vials of 10 percent sucrose solution and 1 vial of DI water)		Daily	X						
COMPLETED EGG PRODUCTION		D114	Daily	X						
Emergence cages	Old O/E container cropped	D111	Daily	X						
	Adult emergence check		Daily	X						
	New pupae placed	D111	M, W, F, Sa, or Su	X						
	DI water (3 vials changed)		Daily	X						
COMPLETED ADULT PRODUCTION		D111	Daily	X						

SCHEDULED COLONY USE
FC102 (Entomology)

93 Year | AA Colony | 8 Room | Sheet No. 2

Action		Data entry	Scheduled and done for given day						
			M	Tu	W	Th	F	Sa	Su
			007	008	009	010	011	012	013
Research use	Adults (emergence cages)	D111	2		2		2		
	Adults (colony BF cages)	D114						4	
	Eggs	D114	5		5		5		
	Larvae	D116		5		5			
	Pupae	D116							
Quality control	Adults (24- to 48-hr-old) to freezer								
Colony shipments		D122							
24- to 48-hour old adults			16						

Julian date	No. of insects being requested	Insect stage
93007	100	24- to 48-hour old ♀'s
	100	4th-Instar larvae
93008	500	♀ Pupae
93009	100	24- to 48-hour old ♀'s
	100	4th-Instar larvae
93010	500	♂ Pupae
93011	100	24- to 48-hour old ♀'s
	100	4th-Instar larvae
93012	1,000	Blood-fed ♀'s (age not critical)

93 Year | AA Colony | 8 Room | Sheet No. 1

COLONY ROOM MAINTENANCE--CULICOIDES
FC 103 (Entomology)

Action		Data entry	Scheduled	Julian dates M 052	Tu 055	W 056	Th 057	F 058	Sa 059	Su 060
FC102 checked/completed use schedule		--	Daily	X						
Light timer On 1:30 a.m. Settings checked Off 2:30 p.m.		--	Daily	X						
Time Started/Completed FC101		D121	Daily	X						
Insect rearing racks	Water jacks in	--	Daily	X						
	Water level floats Down	--	Daily	X						
	Door latches closed	--	Daily	X						
	Exhaust fans on	--	Daily	X						
	High cut out checked	--	Daily	X						
Equipment on/or running	Rearing rack paddles	--	Daily	X						
	Rearing rack lights	--	Daily	X						
	Incubator lights	--	Daily	X						
	Incubator hygrothermograph	--	Daily	X						
	Central air conditioning system	--	Daily	X						
	Floor air conditioner	--	As needed	-						
	Insect light traps	--	Daily	X						
Daily cleanup		--	Daily	X						
Ant security checked		--	Daily	X						
Pupal screens removed		--	Daily	X						
Larval screens removed		--	Th, F, Sat	-						
Hygrothermograph prepared		--	M	X						
Start of new batch recorded		D121	As needed	-						
Drain vent pipes		--	Weekly	X						
Completed production costs		D121	Daily	X						
Check DI water light		--	Daily	X						
Final on departure	Pupal picking pump off	--	Daily	X						
	Deionized water off	--	Daily	X						
	CO_2 turned off, lights off	--	Daily	X						
	Doors and screened Doorways closed	--	Daily	X						

Log Book No.: 017

REQUEST FOR INSECTS
FROM ABADRL'S
INSECT REARING PROGRAM

NOTE: You must provide at least a 3-day notice for requested material; consult with ABADRL's insect rearing personnel on availability.

Date of request: 3/4/93

Name of requestor: Tabachnick For whom: Tabachnick

Laboratory No.: 6030 Phone No.: X3625

Description of insect stages requested:

Eggs _____ Quantity _____
Age _____

* Larvae X Quantity 1000
Age (size)
1st instar (#170 sieve) ___
2d instar (#140 sieve) ___
3d instar (# 80 sieve) ___
4th instar (# 70 sieve) X

Pupae _____ Quantity _____
Age _____
Sex _____

Adults X Quantity 100
Age 24- to 48-hour old
Sex _____
Non blood-fed X
Blood-fed _____

Comments: _____

_____

_____

Date needed: 3/8/93

* larvae are available only on Monday, Tuesday, or Wednesday.

NOTE: You must follow the guidelines of the ABADRL Safety Manual for the transportation, handling, and disposal of all insect stages.

NOTE: You must return all small insectary equipment to ABADRL after use.

Sheet No. 1

QUALITY CONTROL

Adult wing length/adult dry weight

Test date 93224

Colony AA Source date 93222 (24-to 48-hour old)

Microscope 4775092 Objective magnification X40

Calibration 3.85 drum units/mm

Examiner's name Hunt

Fly No.	Drum units	Wing length (mm)	Dry weight (μg)
1	5.96	1.55	99.4
2	5.83	1.51	89.0
3	5.91	1.54	91.7
4	6.12	1.59	108.3
5	5.49	1.43	92.8
6	5.82	1.51	89.6
7	5.92	1.54	96.0
8	6.08	1.58	96.3
9	5.86	1.52	80.6
10	5.97	1.55	104.7
11	5.96	1.55	90.4
12	5.77	1.50	86.7
13	5.96	1.55	92.7
14	5.96	1.55	87.8
15	5.79	1.50	84.0
16	5.92	1.54	92.2
17	5.61	1.46	78.9
18	5.90	1.53	88.0
19	5.66	1.47	77.4
20	5.80	1.51	82.8
21	5.95	1.55	105.2
22	5.76	1.50	76.4
23	5.96	1.55	96.7
24	6.12	1.59	95.7

Mean wing length 1.53 mm

Mean dry weight 91.0 μg

COLONIES – ADULT PRODUCTION

D 111 *(Entomology)*

SHEET NO. 12

$␣ 93 ␣ 8 ␣ 63 ␣ AA ␣ 01 R

YEAR (2) | ROOM (2) | INCUBATOR (2) | COLONY (2) | FOOD TYPE (A) in [4] (2)

POP. [1]	[2]	EMERGENCE TIME [3]				ADULT NUTRITION [4]						PUPAL PRODUCTION [5]									USE [6]			
		Start		End		(B)			(C)			(A)			(B)			(C)			(A)		(B)	
Cage No.	GENER.	Day	Hour (nearest)	Day	Hour (nearest)	Day	Hour (nearest)	Food Type	Day	Hour (nearest)	Food Type	Source Day	CM	No.	Source Day	CM	No.	Source Day	CM	No.	Day	Who	Day	Who
4	2	3	4	3	4	3	4	2	3	4	2	3	1	4	3	1	4	3	1	4	3	2	3	2
1530	00	046	1000	047	0900							R 044	4	0000							R		049	B R
1531	00	046	1000	047	0900							R 045	4	0000							R		049	B R
1532	00	047	0900	048	0800							R 045	4	0000							R		049	B R
1533	00	047	1100	048	0800							R 047	1	0015							R049	8		R
1534	00	048	0800	049	0800							R 047	4	0000							R050	8	051	C R
1535	00	049	0800	050	1000							R 047	4	0000							R		051	C R
1536	00	049	1100	050	1000							R 049	5	0032							R		052	C R
1537	00	049	1100	050	1000							R 049	5	0032							R051	8	051	C R
1538	00	050	1000	051	1000							R 049	4	0000							R		054	A R
1539	00	050	1000	051	1000							R 049	4	0000							R		054	A R
1540	00	051	1000	052	0900							R 049	4	0000							R		054	A R
1541	00	051	1000	052	0900							R 049	4	0000							R		054	A R
1542	00	051	1000	052	0900							R 051	5	0026							R		054	A R
1543	00	051	1000	052	0900							R 051	5	0026							R		054	A R
1544	00	052	0900	053	0800							R 051	4	0000							R		054	A R
1545	00	052	0900	053	0800							R 051	4	0000							R		054	A R
1546	00	052	0900	053	0800							R 052	1	0021							R		053	NF R
1547	00	053	0800	054	1200							R 051	4	0000							R		056	B R
1548	00	053	0800	054	1200							R 051	4	0000							R		056	B R
1549	00	053	0800	054	1200							R 052	4	0000							R		056	B R

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D111)**

␣, |, or ¦ = Space *(hit space bar)*

R = Return *(hit RETURN key)*

¦ = Beginning/Ending of field *(do not space)*

	1	2	3	4	5
K					
LF					
V					

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Form WR-704 (12/79)

USDA-SEA

COLONIES – PHYSICAL PARAMETERS

D112 *(Entomology)*

SHEET NO. ____________

$ b̸ ________ b̸ ________ b̸ ________ b̸ ________ R
YEAR *(2)* ROOM *(2)* ROOM *(2)* ROOM *(2)*

[1]	LARVAL PAN LOCATION AND TEMPERATURE °C [2]																	HOLDING CONDITIONS [3]															
	(A)		(B)		(C)		(D)		(E)		(F)		(G)		(H)			(A)					(B)					(C)					
DAY	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C	Pan No.	°C		Inc. No.	Temp. °C		RH		Inc. No.	Temp. °C		RH		Inc. No.	Temp. °C		RH		
																			High	Low	H	L		High	Low	H	L		High	Low	H	L	
3	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*		*2*	*3*	*3*	*2*	*2*	*2*	*3*	*3*	*2*	*2*	*2*	*3*	*3*	*2*	*2*	
																	R																R
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																	R																R

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D112)**

b̸ , | , or **|** = Space *(hit space bar)*
R = Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

Form ARS-720 (1/86)
(Prev. edition 12/79 may be used)

USDA-ARS

COLONIES – PUPAE PRODUCTION
D113 *(Entomology)*

$b̸ ______ b̸ ______ b̸ ______ R
YEAR *(2)* ROOM *(2)* COLONY *(2)*

[1] DAY *3*	[2] PUPAE *(no./ml)* *3*

[1] DAY *3*	[2] PUPAE *(no./ml)* *3*

[1] DAY *3*	[2] PUPAE *(no./ml)* *3*

For Help key in:
LIST HELP(D113)

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

USDA-SEA

Form WR-705 (12/79)

SHEET NO.______

D113 and D122 are *NOT* related.

NOTE: Italic numerals indicate maximum number of characters to be used.

b̸ , | , or ¦ = Space *(hit space bar)*
R= Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

COLONIES – SHIPMENTS TO OTHER LABORATORIES
D122 *(Entomology)*

$b̸ ______ b̸ ______ R
YEAR *(2)* COLONY *(2)*

[1]	[2]	HOW [3]			COST to ABADRL [4]		
DAY	NAME OF RECIPIENT LAB	Stadium	Counting Method	No.	Shipping Method	Cost	
						$	¢
3	*8*	*1*	*1*	*4*	*8*	*3*	*2*

For Help key in:
LIST HELP(D 122)

	1	2	3	4	5
K					
LF					
V					

K=Keying LF=Listing/Formatting V=Verifying

U.S. Government Printi ice·1980·682·947/319

COLONIES — EGG PRODUCTION
D114 *(Entomology)*

SHEET NO. ____________

$ b̸ ____________ b̸ ____________ b̸ ____________ R
YEAR *(2)* ROOM *(2)* COLONY *(2)*

[1] DAY	[2] CAGES BF		EGG PRODUCTION [3]													EGG USE [4]													HATCH [5]		[6] COLONY FLY USE		
			(A)				(B)				(C)					(A)				(B)				(C)									
	New	Old	Day	CM	No. *(K)*	Inc. No.	Day	CM	No. *(K)*	Inc. No.	Day	CM	No. *(K)*	Inc. No.		Day	CM	No. *(K)*	Who	Day	CM	No. *(K)*	Who	Day	CM	No. *(K)*	Who		Number Counted	Number Hatched	Day	Who	
3	*1*	*2*	*3*	*1*	*4*	*2*	*3*	*1*	*4*	*2*	*3*	*1*	*4*	*2*		*3*	*1*	*4*	*2*	*3*	*1*	*4*	*2*	*3*	*1*	*4*	*2*		*3*	*3*	*3*	*2*	
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NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D114)**

b̸, |, or ❙ = Space *(hit space bar)*
R = Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

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K					
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K=Keying LF=Listing/Formatting V=Verifying

Form WR-706 (12/79)

USDA-SEA

U.S. Government Printi 'ffice-1980-682-942/314

COLONIES — LARVAL PRODUCTION AND IMMATURE USE
D116 *(Entomology)*

SHEET NO. ________

FOOD TYPE ________

$ b̸ ________ b̸ ________ b̸ ________ R
YEAR *(2)* ROOM *(2)* COLONY *(2)*

[1]	LARVAL REARING PAN NUMBERS											HOLDING PANS													[7] LARVAL PANS — Nonstandard Nutrition										[8] USE			
DAY	[2] *DISCARD*					[3] To Holding	[4] *SETUP*					[5] *Setup* Pan No.	Nutrition [6]																						Who	Stadium	C M	No.
	(A)	(B)	(C)	(D)	(E)		(A)	(B)	(C)	(D)	(E)		(A)		(B)		(C)		(D)		(E)		(F)		(A)		(B)		(C)		(D)		(E)					
													Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food	Pan No.	Food				
3	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*1*	*1*	*4*
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NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D116)**

b̸ , | , or | = Space *(hit space bar)*
R = Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

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Form WR-709 (12/79)

USDA-SEA

U.S. Governmen ing Office·1980·682·944/31

COLONIES – PRODUCTION COSTS
D121 *(Entomology)*

SHEET NO. ________

$b̸ ________b̸ ________b̸ ________b̸
YEAR *(2)* ROOM *(2)* COLONY *(2)*

[1]	PERSONNEL USE [2]					[3] MAINTENANCE and SUPPLY MAKEUP and USE					[4] MAINTENANCE and SUPPLY COSTS				
DAY	Initials		Time *(exact minutes)*		T or H	Name Item	No. of	Initials	Time *(exact minutes)*		Name Item	No. of	Cost		
	Scheduled	Done by	Start	End					Start	End			$	¢	
3	*2*	*2*	*4*	*4*	*1*	*8*	*3*	*2*	*4*	*4*	*8*	*3*	*4*	*2*	
						R					R				R
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Form WR - 708 (12/79)

NOTE: Italic numerals indicate maximum number of characters to be used.

For Help, key in: **LIST HELP(D121)**

b̸, |, or | = Space *(hit space bar)*
R = Return *(hit RETURN key)*
¦ = Beginning/Ending of field *(do not space)*

Enter R only at completion of same source day after each field.

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V					

K=Keying LF=Listing/Formatting V=Verifying

USDA-SEA

U.S. Government Printing Office 1980-682-943/315

___ YEAR ___ COLONY ___ ROOM

COLONIES-- LARVAL PRODUCTION

Date	Total larvae ml (Each sieve)	Total larvae ml (All sieves)	Larvae: ml and use			Excess larvae: pan # or discard
			Use			
			Pan #	ml	Pan #	

Year _____ Colony _____ Room _____

Sheet No._____

COLONY OPERATIONS - CULICOIDES
FC 101 (Entomology)

Action		Data entry	Scheduled	Julian dates M	Tu	W	Th	F	Sa	Su
COMPLETED PHYSICAL PARAMETERS		D112	Daily							
Larval pans	Water added		Daily							
	Scum (film) broken up		Daily							
	Discarded	D116	See schedule							
	Setup	D116	See schedule							
Eggs placed on egg hatch pans		D114	W, Th, F							
Egg hatch pans to larval rearing pans		D117	W, Th, F							
Larval rearing pans to pupal rearing		D117	M, Tu, W							
Pupae recovered		D111	M, W, F, Sa, or Su							
Food added to egg hatch, larval rearing, & pupal pans	Bacterial inoculum		At setup							
	"Kalf" medium		At setup							
	"J" medium		W, Th, F							
	Nutrient broth fluid concentrate	D116	Daily							
COMPLETED LARVAL PRODUCTION		D116	Daily							
Colony cages (A-C) & scheduling for	Oviposition dish placed	D114	M, Th, Sa							
	Eggs recorded	D114	Tu, F, Su							
	Egg use recorded	D114	W, Th, F							
	Stored eggs moistened		As needed							
	Cages blood fed	D114	M, W, F							
	Food (2 vials of 10 percent sucrose solution and 1 vial of DI water)		Daily							
COMPLETED EGG PRODUCTION		D114	Daily							
Emergence cages	Old O/E container cropped	D111	Daily							
	Adult emergence check		Daily							
	New pupae placed	D111	M, W, F, Sa, or Su							
	DI water (3 vials changed)		Daily							
COMPLETED ADULT PRODUCTION		D111	Daily							

SCHEDULED COLONY USE
FC102 (Entomology)

Sheet No. _____

_____ Year _____ Colony _____ Room

Action		Data entry	Scheduled and done for given day						
			M	Tu	W	Th	F	Sa	Su
Research use	Adults (emergence cages)	D111							
	Adults (colony BF cages)	D114							
	Eggs	D114							
	Larvae	D116							
	Pupae	D116							
Quality control	Adults (24- to 48-hr-old) to freezer								
Colony shipments		D122							

Julian date No. of insects being requested Insect stage

Year _____ Colony _____ Room _____ Sheet No. _____

COLONY ROOM MAINTENANCE--CULICOIDES
FC 103 (Entomology)

Action		Data entry	Scheduled	Julian dates M	Tu	W	Th	F	Sa	Su
FC102 checked/completed use schedule		--	Daily							
Light timer Settings checked On 1:30 a.m. Off 2:30 p.m.		--	Daily							
Time Started/Completed FC101		D121	Daily							
Insect rearing racks	Water jacks in	--	Daily							
	Water level floats Down	--	Daily							
	Door latches closed	--	Daily							
	Exhaust fans on	--	Daily							
	High cut out checked	--	Daily							
Equipment on/or running	Rearing rack paddles	--	Daily							
	Rearing rack lights	--	Daily							
	Incubator lights	--	Daily							
	Incubator hygrothermograph	--	Daily							
	Central air conditioning system	--	Daily							
	Floor air conditioner	--	As needed							
	Insect light traps	--	Daily							
Daily cleanup		--	Daily							
Ant security checked		--	Daily							
Pupal screens removed		--	Daily							
Larval screens removed		--	Th, F, Sat							
Hygrothermograph prepared		--	M							
Start of new batch recorded		D121	As needed							
Drain vent pipes		--	Weekly							
Completed production costs		D121	Daily							
Check DI water light		--	Daily							
Final on departure	Pupal picking pump off	--	Daily							
	Deionized water off	--	Daily							
	CO_2 turned off, lights off	--	Daily							
	Doors and screened Doorways closed	--	Daily							

Log Book No.: _____

REQUEST FOR INSECTS
FROM ABADRL'S
INSECT REARING PROGRAM

NOTE: You must provide at least a 3-day notice for requested material; consult with ABADRL's insect rearing personnel on availability.

Date of request: _____

Name of requestor: _____ For whom: _____

Laboratory No.: _____ Phone No.: _____

Description of insect stages requested:

Eggs _____
- Quantity _____
- Age _____

* Larvae _____
- Quantity _____
- Age (size)
  - 1st instar (#170 sieve) _____
  - 2d instar (#140 sieve) _____
  - 3d instar (# 80 sieve) _____
  - 4th instar (# 70 sieve) _____

Pupae _____
- Quantity _____
- Age _____
- Sex _____

Adults _____
- Quantity _____
- Age _____
- Sex _____
- Non blood-fed _____
- Blood-fed _____

Comments: _____

_____

_____

Date needed: _____

* larvae are available only on Monday, Tuesday, or Wednesday.

NOTE: You must follow the guidelines of the ABADRL Safety Manual for the transportation, handling, and disposal of all insect stages.

NOTE: You must return all small insectary equipment to ABADRL after use.

Sheet No. _____

QUALITY CONTROL

Adult wing length/adult dry weight

Test date _____

Colony _____ Source date _____ (24-to 48-hour old)

Microscope _____ Objective magnification _____

Calibration _____ drum units/mm

Examiner's name _____

Fly No.	Drum units	Wing length (mm)	Dry weight (μg)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			

Mean wing length _____ mm

Mean dry weight _____ μg

APPENDIX E. PREPARATION, STORAGE, AND SOURCES OF DIETARY FORMULATIONS

Artificial diets supplying proteins, carbohydrates, lipids, sterols, minerals, and vitamins have been developed to provide optimal growth, development, and reproduction of *C. variipennis* (ABADRL, unpublished data). The ingredients of the diets should be purchased in lots or batch items (for example, a 1-year supply of nutrient broth should be obtained at one time) to assure consistent quality. When purchased ingredients arrive, a receiving date and a container-opening date should be placed on the labels of the containers. Ingredients should be stored according to manufacturers' recommendations to preserve quality. The shelf life of each product must be kept in mind. In general, a cool, dry area will suffice for prolonged storage of perishable ingredients. Some dietary formulations (for example, bacterial inoculum, nutrient broth fluid concentrate, sheep blood, and 10 percent sucrose solution) require refrigeration. All new ingredients should be evaluated for toxicity before general use (see *Quality Control*).

Preparation and Storage of Bacterial Inoculum

1. Weigh out 8.0 g of nutrient broth medium using a portable electronic balance.

2. Place the nutrient broth in a 2,000-ml flask containing a Teflon-coated magnet, and add 1,000 ml of DI water.

3. Place the flask on a hot plate/stirrer, and stir at the highest marked setting (that is, near the fast setting) until the nutrient broth is dissolved completely.

4. Pour approximately 100 ml of the solution into each of eleven 125-ml serum bottles, filling each bottle to the curve; cover the mouth of each bottle with a small piece of aluminum foil, label each bottle, and autoclave the bottles for 20 min at 15 lb of pressure and 121 °C.

5. Allow the sterile bottles to cool, transfer 6.0 ml of previously prepared bacterial inoculum into each bottle, and incubate the bottles for approximately 48 hr at 37 °C in the bacteriological incubator.

6. Remove the bottles, and store them in the refrigerator.

Preparation and Storage of "J" Medium

1. Weigh out the following ingredients with a portable electronic balance:
   10.0 g albumin (dried egg white)
   10.0 g brain heart infusion medium
   10.0 g yeast extract medium.

2. Combine the ingredients, mix thoroughly, and store multiple batches in a 1-gal plastic jar at room temperature; retain a portion in a 2-cup plastic container, and store this portion at room temperature for current use.

Preparation and Storage of "Kalf" Medium

1. Grind the coarse high-protein supplement to a fine powder using a hand grinder.

2. Weigh out the following ingredients with a portable electronic balance:
   140.0 g high-protein supplement
   135.0 g alfalfa
   10.0 g albumin (dried egg white)
   10.0 g brain heart infusion medium
   10.0 g yeast extract medium.

3. Combine the ingredients, mix thoroughly, and store multiple batches in a 1-gal plastic jar at room temperature; retain a portion in a 2-cup plastic container and store this portion at room temperature for current use.

Preparation and Storage of Nutrient Broth Fluid Concentrate

1. Add 3,000 ml of DI water into a 8 ¼-qt stainless steel beaker containing a Teflon-coated magnet.

2. Place the beaker on a hot plate/stirrer, set the stirrer at the fastest stirring speed and at the No. 3 heat setting (that is, moderate temperature); simmer the water for approximately 15 min.

3. Slowly add 2.27 kg of nutrient broth medium to the hot DI water.

Caution: Do not allow the nutrient broth solution to boil over because the hot plate/stirrer may be damaged.

Note: The 2.27 kg of nutrient broth medium is the equivalent of one large commercially available container (5-lb jar) of this medium.

4. After the nutrient broth medium has dissolved completely, pour approximately 175 ml of the solution into each of twenty-three 250-ml jars; cover, label, and autoclave the jars for 20 min at 15 lb of pressure and at 121 °C.

5. After cooling, store the jars in the refrigerator.

Acquisition and Storage of Sheep Blood

1. Fill a 50-ml syringe equipped with a 16-gauge hypodermic needle with blood obtained from the jugular vein of a sheep.

2. Place the blood in a sterile 250-ml jar containing 3–5 glass beads.
3. Collect a second 50-ml sample into a second sterile 250-ml jar containing 3–5 glass beads.

Note: Four disease-free sheep are kept at ABADRL to provide blood for insect feeding; the sheep are rotated weekly to prevent anemia or stress. Two jars of sheep blood should provide a 1-wk supply of blood for feeding female adults three times a week.

4. Gently swirl the two jars of blood for 10–15 min to allow the formation of blood clots, and aseptically remove the blood clots with a pair of large forceps.

5. Wipe the jar rim and jar cap with disposable tissue, label the jars with the identification number of the sheep and the current Julian date, and store the jars in the refrigerator at 7 °C.

Caution: Do not store the jars containing sheep blood for more than 10 days because the red blood cells will start to break down.

Preparation and Storage of 10 Percent Sucrose Solution

1. Add 250.0 ml of DI water and 25.0 ml of white granulated sugar into a bottle and shake vigorously to dissolve the sugar.

2. Store the bottle of sugar water in the refrigerator at 7 °C.

Sources of Dietary Formulations

The nutritional ingredients are all commercially available, with the exception of the sheep blood and the microorganisms in the bacterial inoculum. The listed sources are where ABADRL has been able to locate these ingredients and are not provided as a recommendation over any others. The addresses and catalog numbers provided in this list were current at the time of publication but may have since changed.

Albumin (dried egg white), cat. No. 901633: ICN Biochemicals, Inc., 4911 Commerce Parkway, P.O. Box 28050, Cleveland, OH 44128

Alfalfa (entomological grade), cat. No. 11–450: Nutrilite Products, Inc., 19600 6th Street, Lakeview, CA 92353

Bacterial inoculum: Available at ABADRL

Brain heart infusion medium (Difco), cat. No. DF0037–05: VWR Scientific, P.O. Box 39396, Denver, CO 80239

High-protein supplement (Purina Chow Animal Feed #3801, Show Chow 32): Locally available at any feed store

Nutrient broth medium (Difco), cat. No. DF0003–05: VWR Scientific, P.O. Box 39396, Denver, CO 80239

Sheep blood: Available at ABADRL

White, granulated sugar: Locally available at any grocery store

Yeast extract medium (Difco), cat. No. DF0127–05: VWR Scientific, P.O. Box 39396, Denver, CO 80239

APPENDIX F. CONSTRUCTION OF SMALL INSECTARY EQUIPMENT AND ACCESSORIES, AND SOURCES OF SUPPLIES

Construction of Adult Holding Cages

1. Remove and discard the circular section from each lid obtained from new 1-gal cardboard ice cream containers, retain the lid rings, and apply a bead of water-based glue along the seam of the inner section of each container for reinforcement.

2. Use a 9/16-inch-diameter cork borer to punch three equally spaced holes located approximately 2 inches up the wall from the bottom of each container.

Note: The three holes are used to hold the vials of DI water or 10 percent sucrose solution in place.

3. Cut a 2 7/8-inch-diameter hole in the center of the bottom of each container to allow the insertion of an O/E container.

Construction of Dacron Islands

1. Lay out a 4 1/4- by 5-inch cardboard template so that the template is on top of a batt of Dacron material.

2. Trim and discard any dirty, torn, or excess material.

3. Cut 15-inch-long strips across the width of the batt, remove the stitching throughout the material, and cut the strips 4 1/4 inch wide.

Construction of Feeding (Reinforced Silicone) Membranes

1. Cut a 16- by 20-inch (that is, 8 by 10 premarked squares) sheet of Parafilm from a 20-inch by 50-foot roll, fold the 20-inch-wide sheet in half, crease, open the folded sheet, and secure either half section to an 11 1/2- by 18-inch piece of flexible plastic with 1-inch-wide laboratory tape along all 4 sides (fig. 36).

2. Cut two 6- by 6-inch single-layer pieces of material from any reinforced sheer hosiery. Use only the material located between the foot section and the top section of the hosiery.

3. Stretch and secure (that is, anchor) the two 6- by 6-inch pieces of hosiery to the Parafilm about 1 1/2 inches apart from each other with short pieces of 3/4-inch-wide Scotch tape (fig. 37).

Note: The pieces of hosiery must be taut—without any "runs" in the material.

4. Apply a 1/4- to 3/8-inch bead of silicone rubber aquarium sealer along the two sides of each piece of hosiery that are perpendicular to the creased edge of the Parafilm (fig. 38) and fold (that is, close) the Parafilm.

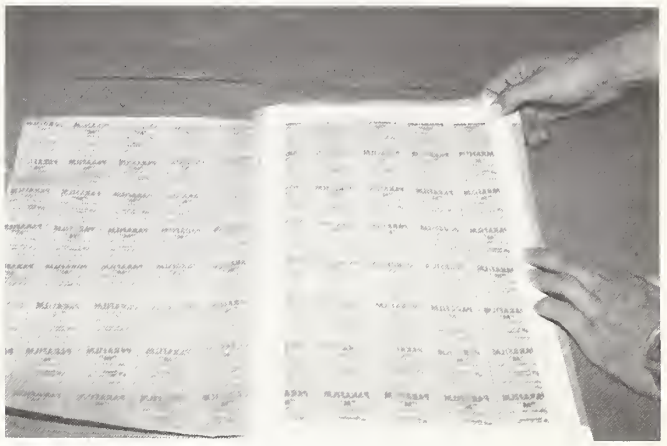

Figure 36. Laboratory tape being applied along the sides of an unfolded half sheet of Parafilm

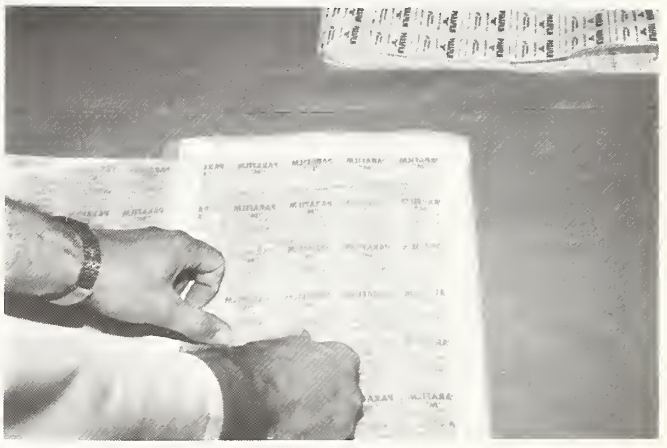

Figure 37. Pieces of hosiery being stretched and secured to the Parafilm with Scotch tape

Figure 38. Bead of silicone sealer being applied along the side of a piece of hosiery

Figure 39. Folded Parafilm containing the hosiery and silicone sealer and being compressed through a roller

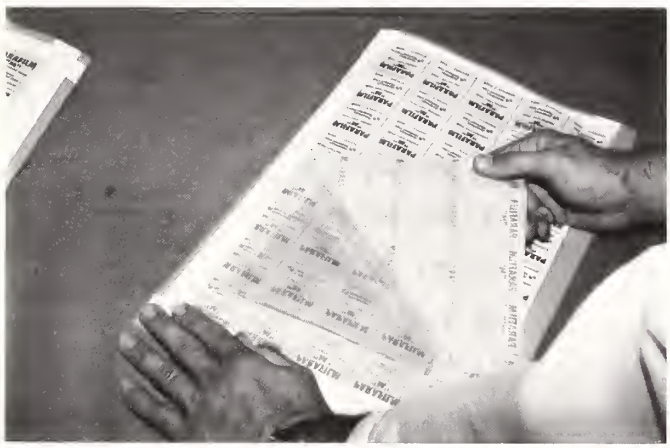

Figure 40. Top half of the Parafilm being removed to expose the hosiery impregnated with silicone sealer so that curing can occur

Figure 41. Two pieces of newly constructed feeding membranes inserted into a bag for autoclaving

5. Insert the folded Parafilm containing the hosiery and silicone sealer into a 12-inch-wide roller typically used for bending sheet metal (fig. 39), adjust the two screws that regulate the spacing between the two rollers to produce a firm pressure (that is, a snug compression) on the folded Parafilm, slowly turn the crank arm to spread the silicone into a uniform layer containing the pieces of hosiery, remove the excess silicone sealer from the rollers and the outside of the Parafilm with disposable tissue, and reinsert the folded Parafilm from the opposite end into the roller.

6. Slightly tighten the two adjusting screws, and crank the Parafilm through the rollers again.

7. Allow the folded Parafilm to cure for 5 min; after 5 min, score along the folded edge of the Parafilm using a single-edge razor blade, slowly remove the top half of the Parafilm (fig. 40), allow the exposed hosiery impregnated with silicone sealer to cure for at least 48 hr, remove the newly constructed feeding membranes from the Parafilm, clean them with mild detergent and warm water by allowing the membranes to soak in this soapy water for at least 24 hr, allow the clean membranes to soak in DI water for at least 24 hr, and then autoclave the membranes (fig. 41).

Note: Removing the Parafilm to expose the hosiery during the 5-min curing process creates the rough texture that simulates the skin of sheep and enhances insect feeding.

8. Cut each 6- by 6-inch piece of feeding membrane into four 2 ½- by 2 ½-inch pieces for use with the water-jacketed glass feeders, and discard the excess material.

Note: For further information, refer to Davis et al. (1983).

Construction of Flotation Screens

1. Cut out 5- by 21-inch and 3- by 21-inch sections from ½-inch galvanized hardware cloth.

2. Dip all edges of the flotation screens into a plastic coating diluted with lacquer thinner.

Construction of Flotation Tub

1. Drill a ½-inch-diameter hole approximately 2 inches from the top edge in any corner of a 21- by 21- by 11-inch galvanized tub.

2. Solder a gradual-bend, 8-inch-long, ½-inch-diameter copper tubing around the hole, and solder a brace between the tub and bottom outlet of the drain tube (fig. 42).

Figure 42. Closeup of the drainage tube attached to the flotation tub

Construction of Holding-Cage Screens

1. Lay out a swatch of fine-mesh nylon organdy.

2. Use large (9⅛-inch diameter) and small (5⅛-inch diameter) cardboard templates to draw circles of each size on the material.

3. Cut out each circle.

4. In a fume hood, apply clear household cement, adhesive, or Dritz Fray Check along the edges of the holding cage screens to prevent fraying of the material.

Construction of Larval Measuring Tube

1. Cut a 15-ml section from a ½-inch-diameter plastic buret.

2. Attach a ½-inch-diameter piece of 0.0035-size-opening stainless steel screen (for example, cut from a used or damaged No. 170 Tyler screen scale equivalent sieve) to one end of the measuring tube using a silicone adhesive.

Construction of Oviposition Papers

1. Lay out sheets of 20- by 20-inch grade No. 595 filter paper.

2. Use the 2¾-inch-diameter cardboard template to draw circles.

3. Cut out each circle.

Note: This cutting can be avoided by using Whatman Grade #2 7-cm-diameter qualitative filter paper disks instead of the sheets.

Construction of O/E Containers

1. Use an industrial lathe to cut ½-inch pieces off of a ⅛-inch-thick, 3-inch-diameter acrylic tubing.

2. Draw 3 ½-inch-diameter circles on an easily handled (for example, 2-ft-wide by 2-ft-long) ⅛-inch-thick acrylic sheet.

3. Cut out each circle with an industrial band saw, and smooth (cut) the edges of a stacked group of circular pieces with an industrial lathe.

4. In a fume hood, center the tubing sections on the circular bases, and apply an acrylic solvent cement to each O/E container.

Construction of Paddles

1. Connect a heating chamber to a temperature-regulating device (fig. 43), set the device at the highest temperature setting (250 °F), and wait approximately 10 min or until the continuous clicking sound stops and the red light goes off.

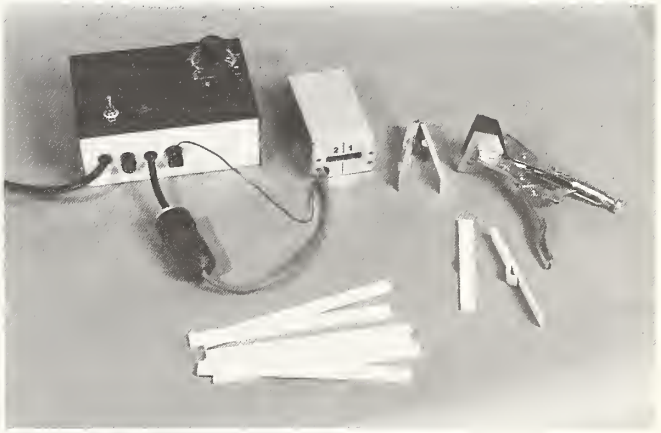

Figure 43. Heating chamber (right) connected to a temperature regulating device (left)

2. Insert a 5/8- by 8 3/8- by 1/8-inch plastic strip into slot No. 1 of the heating chamber (fig. 44), and wait until the plastic strip sags (30–60 sec).

3. Immediately remove the strip, place it in a mold, and compress the mold for approximately 1 min or until the mold and the strip cool (fig. 45).

Caution: Allow the plastic strip to cool completely or else it will spring out of shape.

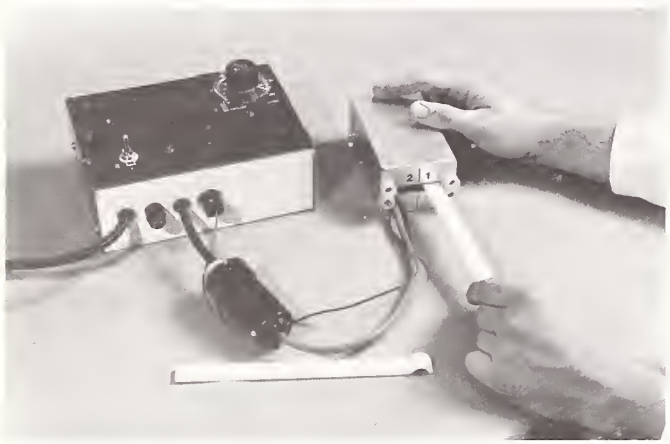

Figure 44. Plastic strip being inserted into slot No. 1 of the heating chamber

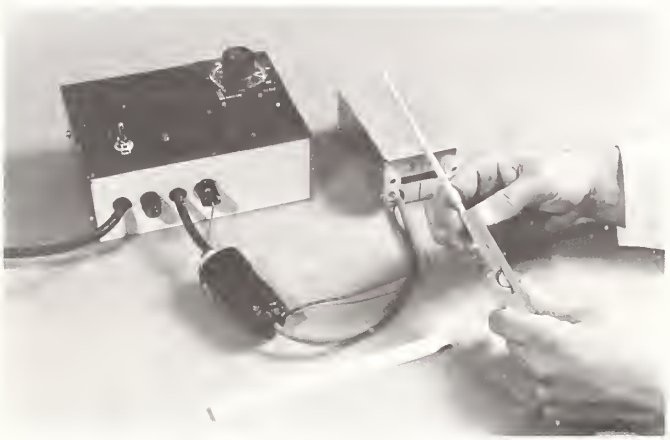

Figure 45. Plastic strip being reshaped in a mold

4. Insert the straight end of the plastic strip through slot No. 2 of the heating chamber until 1 inch of the strip extends from the chamber (fig. 46), and heat until the strip sags (30–60 sec).

5. Remove the plastic strip, place it in the mold, bend the straight end 180° at the end of the form (fig. 47), and squeeze the clamp over the plastic strip and the form (fig. 48) for approximately 1 min or until the form and plastic strip cool.

Caution: Allow the plastic strip to cool completely or else it will spring out of shape.

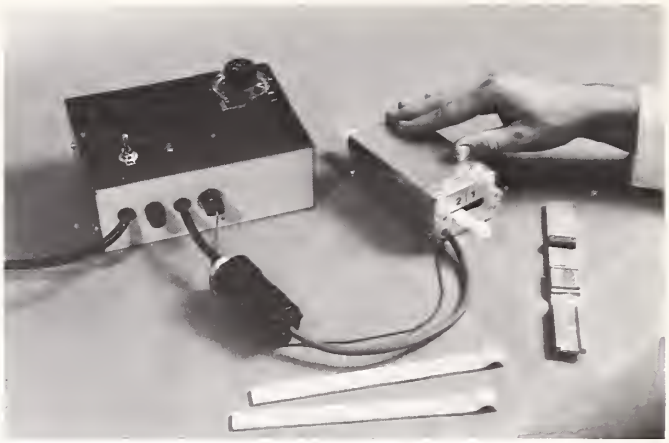

Figure 46. Plastic strip being inserted into slot No. 2 of the heating chamber

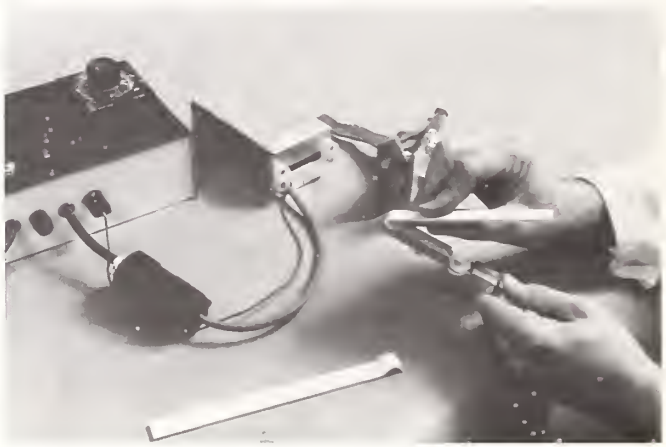

Figure 47. Plastic strip being folded on the mold

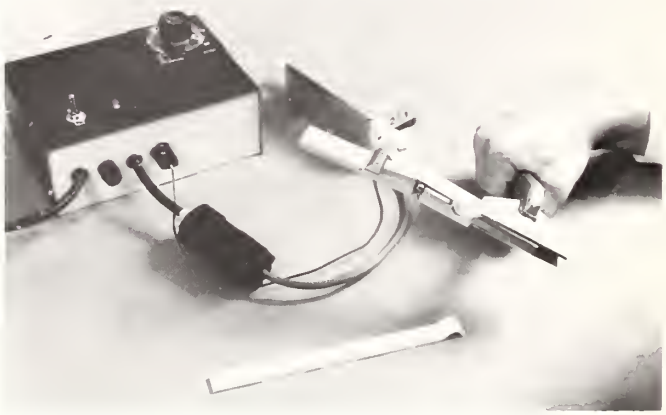

Figure 48. Plastic strip being reshaped in the mold

Construction of Pupal Measuring Tube

1. Cut a 15-ml section from a 1/2-inch-diameter plastic buret.

2. Attach a 1/2-inch-diameter piece of 0.0083-size-opening stainless steel screen (for example, cut from a used or damaged No. 70 Tyler screen scale equivalent sieve) to one end of the measuring tube with a silicone adhesive.

Construction of Vial Wicks

1. Cut 1 ½-inch-long No. 3 dental cotton rolls into 1-inch-long pieces.

Construction of Vials Used for DI Water or 10 Percent Sucrose Solution

1. Place two No. 19 rubber bands along the length of a 2-dram (8-ml) vial, and make the curved ends of the rubber bands go around the bottom of the vial (fig. 49).

2. Pull on the two rubber bands to stretch them so that the curved ends remain in place, and continuously wrap a No. 64 rubber band around the bottom of the two No. 19 rubber bands and vial (fig. 50).

Figure 49. Two No. 19 rubber bands being placed around a vial that will hold water or sugar water

Figure 50. No. 64 rubber band being wrapped around the two No. 19 rubber bands and a vial

Sources of Supplies

The listed sources are where ABADRL has been able to locate supplies and are not provided as a recommendation over any others. The catalog numbers, item numbers, and addresses in this list were current at the time of publication but may have since changed.

Acrylic solvent cement, Weld-On 4 brand: Industrial Polychemical Service, 17116 S. Broadway, Bardena, CA 90247

Aquarium algae scraper: Locally available at any pet store

Dacron batts (polyester fiberfill), cat. No. 08014472: Van Waters & Rogers, 4300 Holly Street, Denver, CO 80216

Dental cotton rolls (1 ½-inch long, large size, No. 3), cat. No. 2729: HealthCO, 8745 E. Orchard Drive, Englewood, CO 80111

Filter paper, cat. No. 28431–009 (grade No. 595) or cat. No. 28455–062 (grade No. 2): VWR Scientific, P.O. Box 39396, Denver, CO 80239

Fine-mesh nylon organdy: Locally available at any fabric store

Ice cream containers (1-gal, cardboard, plastic-lined, 6 ¾-inch diameter), cat. No. 0000–1–30227, item No. 1227: Ricker Brothers, Inc., 220 North Hawes, Fort Collins, CO 80524

Insect rearing pan (injection-molded unplasticized PVC): Plastic Design and Manufacturing Co., 1155 S. Cherokee, Denver, CO 80223

Parafilm, cat. No. 52859–079: VWR Scientific, P.O. Box 39396, Denver, CO 80239

PVC plastic (buy sheets and cut them for use on stirring paddles): Available at any plastic supply company

Reinforced sheer hosiery, model 415L Barely There: Hanes Hosiery, P.O. Box 202, Winston-Salem, NC 27102

Roller, model PR–10A: Roper Whitney Co., 2833 Huffman Blvd., Rockford, IL 61103

Rubber bands (No. 19 and No. 64): Locally available at any office supply store

Scotch tape, model 810: 3M Commercial Office Supply Division, St. Paul, MN 55144

Silicone sealer (Hartz Silicone Rubber Aquarium Sealer): The Hartz Mountain Corporation, 700 Frank E. Rodgers Blvd., South, Harrison, NJ 07029

Stainless steel metal bars: Available at any steel fabricating company

Sterile cotton, cat. No. 23530–003: VWR Scientific, P.O. Box 39396, Denver, CO 80239

Vials for water and 10 percent sucrose solution (2-dram or 8-ml with lip), cat. No. 66010–107: VWR Scientific, P.O. Box 39396, Denver, CO 80296